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Radiometric age validation of Atlantic tarpon, *Megalops atlanticus*

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Abstract–An improved radiometric aging technique was used to examine annulus-derived age estimates from otoliths of the Atlantic tarpon, *Megalops atlanticus*. Whole otoliths from juvenile fish and otolith cores, representing the first 2 years of growth, from adult fish were used to determine ^{210}Pb and ^{226}Ra activity; six age groups consisting of pooled otoliths and nine individual otolith cores were aged. This unprecedented use of individual otolith cores to determine age was possible because of improvements made to the ^{226}Ra determination technique. The disequilibria of ^{210}Pb:^{226}Ra for these samples were used to determine radiometric age. Annulus-derived age estimates did not agree closely with radiometric age determinations. In most cases, the precision (CV≤12%) among the otolith readings could not explain the differences. The greatest radiometric age was 78.0 yr for a 2045-mm-FL female, where the radiometric error encompassed the annulus-derived age estimate of 55 yr by about 4 yr. The greatest radiometric age for males was 41.0 yr for a 1588-mm-FL tarpon, where the radiometric error encompassed the annulus-derived age estimate of 32 yr by 1 yr. Radiometric age determinations in this study indicated that the interpretation of growth zones in Atlantic tarpon otoliths can be difficult, and in some cases may be inaccurate. This study provides conclusive evidence that the longevity of the Atlantic tarpon is greater than 30 years for males and greater than 50 years for females.

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The Atlantic tarpon (*Megalops atlanticus*) is believed to be a long-lived fish. The strongest evidence is from a captive female Atlantic tarpon that lived to at least 63 years when it died in 1998 at the John G. Shedd Aquarium in Chicago, Illinois.[1] Life in the aquarium, however, is difficult to compare with life in the natural environment. A similar longevity of 55 years was estimated from growth zones in sagittal otoliths of a wild female tarpon (Crabtree et al., 1995). The annual periodicity of the growth zones used to estimate age was validated by using oxytetracycline up to an age of 9 yr in captive fish (Crabtree et al., 1995), but extrapolation of these results to older, wild fish, however, is fallible. In addition, wild Atlantic tarpon are highly migratory and inhabit waters that vary considerably in salinity (0 to 43 ppt) and temperature (17° to 37°C; Zale and Merrifield, 1989; Nichols[2]). It is uncertain what affect these changing conditions have on the formation of growth zones in the otoliths, but periods of stress have been shown to halt otolith growth (Campana, 1983). Furthermore, growth slows with age and growth zones become increasingly compressed, rendering otolith growth zones difficult to interpret. To circumvent the potential problems of otolith interpretation and to independently determine age, an application of the radiometric aging technique by using the ^{210}Pb:^{226}Ra disequilibria in sagittal otolith cores (Campana et al., 1990) of Atlantic tarpon was performed, and the results are discussed in the context of existing estimated growth parameters.

Materials and methods

Pooled and individual otolith cores from Atlantic tarpon were analyzed for ^{210}Pb and ^{226}Ra to determine age. Annulus-derived age estimates were based on six independent otolith readings determined by Crabtree et al. (1995) and resulted in a coefficient of variation of less than 12%. For radiometric age determination, otoliths were pooled into six groups based on annulus-derived age estimates, sex, and collection date. In addition, nine individual otoliths were analyzed. The age groups were selected to cover the full range of annulus-derived age estimates. Selected age groups had a narrow age range and members of the group were collected within a 6-month period. Otoliths of adult age groups were cored to the first two years of growth and otoliths of the youngest age groups were analyzed whole because they were similar in size to cores from adult fish. Because 2-yr cores were large and the activity of ^{226}Ra was relatively high (~10 to 100 times higher than usual; Andrews et al., 1999b), single otoliths were analyzed with the radiometric aging technique.

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[1] Pamper, K. 1999. Personal commun. John G. Shedd Aquarium, 1200 S. Lakeshore Dr., Chicago, IL 60605.

[2] Nichols, K. M. 1994. Age and growth of juvenile tarpon, *Megalops atlanticus*, from Costa Rica, South Carolina and Venezuela. Senior thesis, Univ. South Carolina, Columbia, SC 29208, 52 p.

Coring the adult otoliths required establishing a target size and weight that closely approximated that of an otolith from a 2-year-old fish. Dimensions and weights of otoliths from four juvenile fish aged 2 years were recorded and averaged; the resultant dimensions were approximately 12 mm long by 6 mm high by 1 mm thick and a weight of 0.1 g. Each otolith from each adult age group was sculpted into this shape and weight by being hand-ground on a Buehler Ecomet® III lapping wheel. All samples were cleaned of any adhering contamination by following specific procedures described elsewhere (Andrews et al., 1999b). These clean samples were placed in acid-cleaned 100-mL Teflon® PFA Griffin beakers and dried at 85°C for 48 h.

A detailed protocol describing sample preparation, chromatographic separation of ^{226}Ra from barium and calcium, and analysis of ^{226}Ra using thermal ionization mass spectrometry (TIMS) is described elsewhere (Andrews et al., 1999b). Only an overview of the ^{226}Ra procedures is given here with details on the determination of ^{210}Pb activity. Because the levels of ^{226}Ra and ^{210}Pb typically found in otoliths were extremely low (from femtograms [10^{-15} g] for ^{226}Ra and attograms [10^{-18} g] for ^{210}Pb) and because of the great potential for contamination from calcium, barium, and lead, trace-metal clean procedures and equipment were used throughout sample preparation, separation, and analysis. All acids used were double distilled (GFS Chemicals®) and dilutions were made with Millipore® filtered Milli-Q water (18 MΩ/cm).

To determine ^{226}Ra activity with thermal ionization mass spectrometry (TIMS), the sample must be clean of naturally occurring organics (such as otolin). Organic residues elevate background counts in the ^{226}Ra region and increase the analytical uncertainty during TIMS analysis. Dried and weighed samples were dissolved in beakers on hot plates at 90°C by adding 8N HNO_3 in 1–2 mL aliquots. Alternation between 8N HNO_3 and 6N HCl, with an aqua regia transition, several times resulted in complete sample dissolution. The dried sample, after dissolution, formed a yellowish foam. To further reduce any remaining organics, and to put the residue into the chloride form required for the ^{210}Pb activity determination procedure, the samples were redissolved in 1 mL 6N HCl and taken to dryness five times at ~90°C. A whitish residue indicated that a sufficient amount of the organics had been removed. These samples were used to determine ^{210}Pb activity prior to TIMS analysis.

Determination of ^{210}Pb Activity

To determine ^{210}Pb activity in the otolith samples, the alpha-decay of ^{210}Po was used as a daughter proxy for ^{210}Pb. To ensure that activity of ^{210}Po was due solely to ingrowth from ^{210}Pb, the time elapsed from capture to ^{210}Pb determination was greater than 2 yr. Samples prepared for ^{210}Po analysis were spiked with ^{208}Po, a yield tracer. The amount of ^{208}Po added was estimated on the basis of observed ^{226}Ra levels in otoliths of juvenile tarpon. This amount was adjusted to five times the expected ^{210}Po activity in the otolith sample to reduce error in the ^{210}Pb activity determination. The spiked samples were redissolved in approximately 50 mL of 0.5N HCl on a hot plate at 90°C covered with a watch glass. The ^{210}Po and ^{208}Po-tracer were autodeposited for 4 hours onto a silver planchet (Flynn, 1968). The activities of these isotopes were determined by using alpha-spectrometry on the plated samples. Quantification of the ^{210}Po was made by subtracting a detector blank and reagent counts from each peak region-of-interest, by multiplying the ^{210}Po:^{208}Po count ratio by the known ^{208}Po activity, and by correcting for decay back to the time of plating. To attain sufficient counts, samples were counted for 21–23 d. The solution remaining after polonium plating was dried and saved for ^{226}Ra analyses.

Determination of ^{226}Ra Activity

To prepare the samples for ^{226}Ra activity determination with TIMS, each sample was spiked with ^{228}Ra, a yield tracer, and a newly developed ion-exchange separation technique was used to isolate radium from calcium and barium (Andrews et al., 1999b). The final samples were processed by using TIMS and the measured ratios of ^{226}Ra:^{228}Ra were used to calculate ^{226}Ra activity. The analysis of unspiked otolith samples indicated that ^{228}Ra was not present in measurable quantities in otoliths of juvenile fish, and no adjustment was necessary for the measured ^{226}Ra:^{228}Ra ratio in spiked samples.

Radiometric age determination

To assess the feasibility of applying the radiometric aging technique to Atlantic tarpon, uptake of ^{210}Pb and ^{226}Ra was assessed in otoliths from juvenile fish. Because the age of juvenile Atlantic tarpon is better constrained than that of adults, age was determined by using ^{210}Pb:^{226}Ra disequilibria in whole otoliths from juvenile fish. For the juvenile otolith samples, the age determined would be higher than expected if a significant amount of exogenous ^{210}Pb was incorporated into the otolith.

Age was estimated from the measured ^{210}Pb and ^{226}Ra activities (Eqs. 1 and 2). Because the activities were measured from the same sample, the calculation was independent of sample mass. For adult samples, where estimated age was greater than that of the 2-year-old core, radiometric age was calculated as follows with an equation derived from Smith et al. (1991) to compensate for the ingrowth gradient of ^{210}Pb:^{226}Ra in the otolith core,

$$t_{age} = \frac{\ln\left(\dfrac{1-\dfrac{A^{210}\mathrm{Pb}_{tc}}{A^{226}\mathrm{Ra}_{\mathrm{TIMS}}}}{(1-R_0)\left(\dfrac{1-e^{-\lambda T}}{\lambda T}\right)}\right)}{-\lambda}+T, \qquad (1)$$

where t_{age} = the radiometric age at the time of capture;
$A^{210}Pb_{tc}$ = the ^{210}Pb activity corrected to time of capture;
$A^{226}Ra_{TIMS}$ = the ^{226}Ra activity measured with TIMS;
R_0 = the activity ratio of ^{210}Pb:^{226}Ra initially incorporated;
λ = the decay constant for ^{210}Pb (ln(2)/22.26 yr); and
T = the core age (2 yr).

The radiometric age calculation for the juvenile age-group was determined by iteration of an equation derived from Smith et al. (1991),

$$\frac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}} = 1-(1-R_0)\left(\frac{1-e^{-\lambda t_{age}}}{\lambda t_{age}}\right), \qquad (2)$$

where all equation components were as defined above. A radiometric age range, based on the analytical uncertainty, was calculated for each sample by applying the calculated error for ^{210}Pb and ^{226}Ra activity determinations to the measured ^{210}Pb:^{226}Ra. Calculated error included the standard sources of error (i.e. pipetting, spike, and calibration uncertainties), alpha-counting statistics for ^{210}Pb (Wang et al., 1975), and an analysis routine used to run ^{226}Ra samples on the thermal ionization mass spectrometer (Andrews et al., 1999b).

Age estimate accuracy

To compare annulus-derived age with radiometric age, a plot of the annulus-derived age estimate and measured ^{210}Pb:^{226}Ra activity ratio was compared graphically to the expected ^{210}Pb:^{226}Ra activity ratio from ingrowth. This comparison included a graphical compensation for the ^{210}Pb:^{226}Ra gradient in the core sample. This model assumed a linear mass-growth rate for the first two years of growth. Annulus-derived age range and the analytical uncertainty of the activity ratio were plotted with each data point. A direct comparison of annulus-derived age and radiometric age was made in a plot where a regression of the data was compared to a line of agreement or slope of one. A paired two-sample *t*-test was used to determine if a significant difference existed between the age estimates.

Von Bertalanffy growth functions were fitted to the radiometric ages with FISHPARM software (Saila et al., 1988) and plotted with the annulus-derived ages and growth functions from Crabtree et al. (1995) for a visual comparison. High and low radiometric age and the size range of each sample were plotted for each data point. No statistical comparison was made between the growth functions because of the low number of samples and wide confidence interval for each parameter.

Results

Six age groups and nine individual otolith cores were selected for radiometric analyses (Table 1). Of the age group samples, three male samples and three female samples were selected to span the estimated age range of each sex. Male age groups were 2–4 yr, 18–21 yr, and 31–36 yr. Female age groups were 3–4 yr, 15–24 yr, and 48–50 yr. For each age group the capture dates were within a 6-month period, except the 2–4 yr male age group where the period spanned 7 months. Individual otolith core samples ranged in annulus-derived age from 13 yr to 32 yr for males and 35 yr to 55 yr for females. The greatest annulus-derived ages were for females; the oldest female was estimated to be 55 yr and the oldest male was 36 yr. Fork length was lowest for the juvenile age groups and did not overlap with older age groups. There was some overlap between the middle and old age groups. Males were typically smaller than females; the largest male was 1620 mm FL and the largest female was 2045 mm FL. The number of otoliths used in each age group ranged from 4 to 11 with the fine-cleaned sample weight ranging from 0.3314 to 1.0718 g. The individual core samples ranged in weight from 0.0884 g to 0.1366 g. These samples are the lowest weights and the first individual otolith cores ever used for radiometric age determination.

Activities of ^{210}Pb and ^{226}Ra were determined and combined to form an activity ratio for each sample (Table 2). The ^{210}Pb activity spanned a wide range and increased from juveniles to adults by as much as 88 times. The lowest activities were for the juvenile samples (0.003 and 0.005 disintergrations per gram [dpm/g]) and the highest activity was 0.265 dpm/g (±6.0%) for the largest male (1620 mm FL). The activity of ^{226}Ra varied by an order of magnitude and ranged from 0.044 dpm/g (±1.53%) to 0.401 dpm/g (±1.02%). Calculated ^{210}Pb:^{226}Ra ranged, as predicted, between 0 and 1; the lowest activity ratios were for the juvenile samples and the highest were for large adults. All low and high ratios were within the limits of 0 to 1 (values >1 are mathematically undefined; Eqs. 1 and 2) except for the largest female (2045 mm FL), which had an upper limit that exceeded 1. Radiometric age of the juvenile samples was very close to the expected age (Table 3). Exogenous ^{210}Pb was, therefore, either not present or present in negligible quantities. This was inferred to be true for the core, or juvenile region, of adult Atlantic tarpon otoliths.

Comparison of annulus-derived ages and radiometric ages indicated there were differences in the aging results for each technique (Table 3). In most cases, the precision among the otolith readings could not explain the differences. In four cases, the different age estimates overlapped, but the extent of overlap was at the extreme of the age range. The lowest radiometric ages were one year for the samples from juvenile fish and the highest was 78.0 yr for the largest adult female.

A graphical comparison of the expected ^{210}Pb:^{226}Ra disequilibria from ingrowth with the measured ^{210}Pb:^{226}Ra indicated there was variation in the measured results above and below what was expected (Fig. 1). The low radiometric age for the juvenile age-groups indicated that uptake of exogenous ^{210}Pb (R_0; Campana et al., 1990) by juveniles was insignificant. Therefore, the best model for ingrowth of ^{210}Pb from ^{226}Ra in tarpon otoliths was for R_0 = 0.0.

Table 1

Detailed summary of data for pooled otolith age groups and single otoliths from *Megalops atlanticus*. Age range of age groups is based on annulus-derived age estimates. Capture dates and average fork length are listed for comparison. Age groups consisted of 4 to11 otolith cores and amounted to ~1 g. Single otolith cores weighed close to 0.1 g.

Age-group or single otolith age (yr)	Capture date or range	Fork length (mm ±SD)	Number of otoliths	Sample weight (g)
Male				
2–4[1]	7 Mar–16 Oct 1989	568 ±80	7	0.6786
13	22 Jun 1989	1499	1	0.1129
17	4 Jul 1989	1452	1	0.1124
18–21	1 May–25 Jul 1989	1442 ±78	10	1.0498
31–36	12 Jun–7 Jul 1990	1532 ±73	4	0.3480
32	6 Jun 1989	1588	1	0.1279
32	19 Jul 1992	1620	1	0.1220
Female				
3–4[1]	24 Jan–15 Jul 1989	583 ±59	9	0.8775
15–24[2]	6 Jun–12 Aug 1989	1641 ±92	11	1.0718
35	4 Jul 1989	1613	1	0.1002
36	12 May 1992	1780	1	0.1331
37	12 Jun 1993	1950	1	0.1167
44	2 Sep 1991	2040	1	0.1366
48–50	20 Apr–20 Jun 1989	1708 ±110	4	0.3314
55	4 Mar 1991	2045	1	0.0884

[1] Whole juvenile otoliths.

[2] Ten otoliths ranging in age from 21 to 24 yr; one 15-yr-old otolith was erroneously included in this age group.

Table 2

Radiometric results for each *Megalops atlanticus* age group and single otolith samples. Activities are expressed as disintegrations per minute per gram (dpm/g).

Age-group or single otolith age (yr)	Sample weight (g)	^{210}Pb (dpm/g) ± % error[1]	^{226}Ra (dpm/g) ± % error[2]	^{210}Pb:^{226}Ra activity ratio	^{210}Pb:^{226}Ra low	^{210}Pb:^{226}Ra high
2–4[3]	0.6786	0.005 ±6.7	0.258 ±1.03	0.017	0.016	0.019
13	0.1129	0.043 ±12.1	0.085 ±1.52	0.507	0.439	0.577
17	0.1124	0.035 ±13.6	0.061 ±1.61	0.568	0.483	0.656
18–21	1.0498	0.072 ±3.4	0.246 ±1.01	0.292	0.280	0.305
31–36	0.3480	0.094 ±5.3	0.181 ±1.25	0.520	0.486	0.555
32	0.1279	0.031 ±13.5	0.044 ±1.53	0.712	0.607	0.821
32	0.1220	0.265 ±6.0	0.401 ±1.02	0.663	0.617	0.710
Female						
3–4[3]	0.8775	0.003 ±5.7	0.217 ±1.06	0.015	0.014	0.016
15–24	1.0718	0.059 ±3.9	0.163 ±1.07	0.362	0.345	0.381
35	0.1002	0.119 ±8.2	0.299 ±1.35	0.397	0.360	0.435
36	0.1331	0.086 ±8.5	0.144 ±2.24	0.597	0.534	0.663
37	0.1167	0.126 ±8.0	0.243 ±1.18	0.518	0.471	0.567
44	0.1366	0.113 ±7.4	0.170 ±1.33	0.667	0.609	0.726
48–50	0.3314	0.148 ±4.5	0.266 ±1.19	0.555	0.524	0.587
55	0.0884	0.086 ±12.2	0.094 ±1.42	0.909	0.787	1.035

[1] Calculation based on standard deviation of ^{210}Pb activity; Wang et al., 1975.

[2] Calculation based on TIMS analysis routine (±1 SE).

[3] Whole juvenile otoliths.

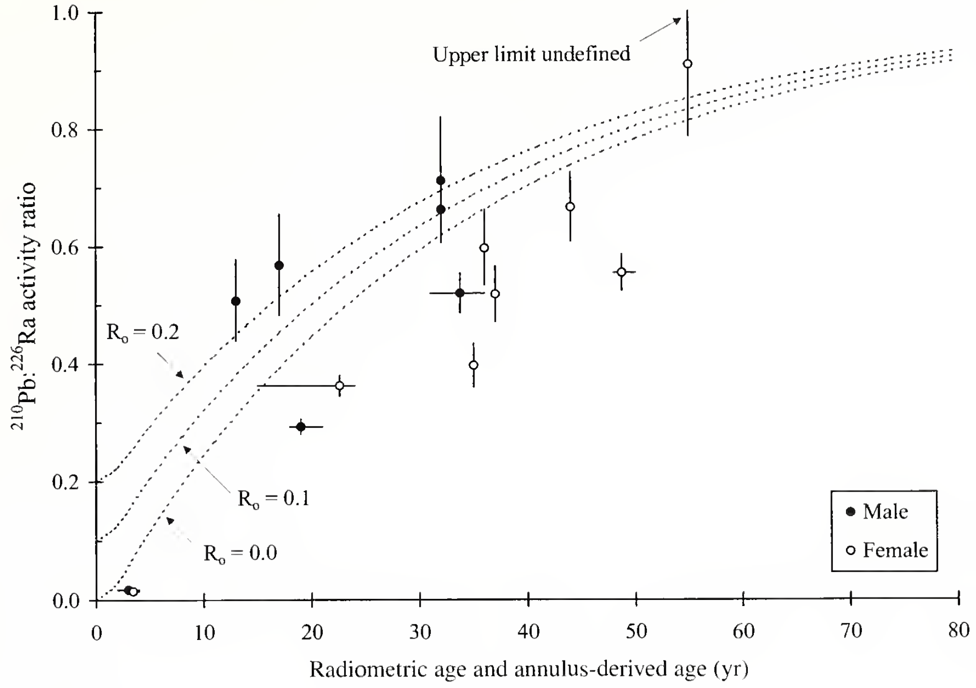

Figure 1

Expected ^{210}Pb:^{226}Ra ingrowth curves and observed ^{210}Pb:^{226}Ra activity ratios for male and female Atlantic tarpon (*Megalops atlanticus*) individuals and age groups. Expected ingrowth curves represent initial uptake ratios (R_0) of 0.0, 0.1, and 0.2. Expected ^{210}Pb:^{226}Ra ingrowth during early otolith growth (until core-age) is based on a linear mass-growth model. After core growth, the secular equilibrium model for ^{210}Pb:^{226}Ra resumes and continues to unity. Data points are annulus-derived ages (average age for age groups) plotted against measured ^{210}Pb:^{226}Ra activity ratios. Vertical bars represent analytical uncertainty of ^{210}Pb and ^{226}Ra measurements. Horizontal bars represent the range of annulus-derived ages for age groups. The upper limit of the ^{210}Pb:^{226}Ra ratio for the oldest female exceeded 1.0; therefore, high radiometric age is mathematically undefined (Eq. 2).

Table 3

Comparison of annulus-derived ages and radiometric ages for *Megalops atlanticus*. The mean age of each group is based on annulus-derived age estimates. The radiometric age range is based on low and high activity ratios from analytical uncertainty calculations. The radiometric age calculations are based on the measured ratio of ^{210}Pb:^{226}Ra.

Age-group or single otolith age (yr)	Mean annulus-derived age (yr)	Radiometric age range (yr)	Radiometric age (yr)	Age-group or single otolith age (yr)	Mean annulus-derived age (yr)	Radiometric age range (yr)	Radiometric age (yr)
Male				Female			
2–4	3.0	1.1–1.2	1.1	3–4	3.4	0.9–1.1	1.0
13	13	19.6–28.7	23.7	15–24	22.6	14.6–16.4	15.5
17	17	22.2–35.3	28.0	35	35	15.3–19.3	17.2
18–21	19.0	11.5–12.7	12.1	36	36	25.5–35.9	30.2
31–36	33.8	22.4– 27.0	24.6	37	37	21.5–27.8	24.5
32	32	31.0–56.2	41.0	44	44	31.2–42.6	36.3
32	32	31.8 – 40.8	35.9	48–50	48.8	24.8–29.4	27.0
				55	55	50.6–undefined[1]	78.0

[1] Upper radiometric age limit for the 78-yr female is undefined because the ^{210}Pb:^{226}Ra ratio exceeded 1.0.

Hence, radiometric age was determined on the basis of measured activity ratios and no adjustment for exogenous ^{210}Pb was necessary. When the radiometric and annulus reading (±12% CV) age ranges were considered graphically, most of the data points still did not agree with the expected ingrowth curve (R_0=0.0).

A direct comparison of annulus-derived age with radiometric age indicated that the ages were evenly distributed on either side of a line of agreement and that the slope of the regression (slope=0.915) was close to 1 (Fig. 2). Radiometric age was not statistically different from the annulus-derived age estimates (paired two-tailed t-test, df=14, t=0.4181, P=0.6822). The coefficient of determination was low (adjusted r^2=0.55; Kvålseth, 1985) and the power of the test to detect significant differences was low because of the variability of the data and the small sample size. The analytical uncertainty associated with radiometric age determination or the reading range of the annulus-derived age encompassed the line of agreement for only four samples. The sample with the closest agreement was a female (1780 mm FL) whose annulus-derived age was 36 years. The annulus-derived age estimates of the juvenile age groups were high by 1.9 and 2.4 years. For older age groups and individual cores, annulus-derived age was higher than radiometric age by as much as 21.8 years and lower than radiometric age by as much as 23 years. Otolith sections that had radiometric and annulus-derived age estimates that differed considerably were photographed to illustrate the difficulty in aging otoliths (Fig. 3).

Von Bertalanffy growth functions fitted to the radiometric ages of males and females (Figs. 4 and 5) were similar to annulus-derived growth functions determined by Crabtree et al. (1995). However, the low number of radiometric data points resulted in large confidence intervals (±95%) for growth model parameters. Radiometric-age growth parameters for males indicated an asymptotic length (L_∞) of 1550 ±83 mm FL and a growth coefficient (k) of 0.19 ±0.12 (Fig. 4). Radiometric-age growth parameters for females indicated an L_∞ of 2030 ± 227 mm FL and a growth coefficient (k) of 0.08 ±0.04 (Fig. 5).

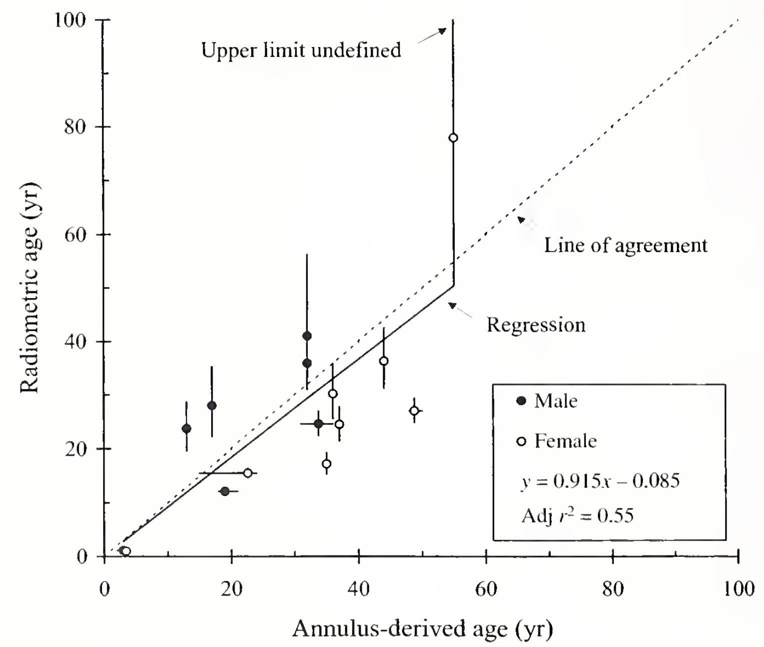

Figure 2

Comparison of male and female Atlantic tarpon (*Megalops atlanticus*) annulus-derived ages and radiometric ages. A linear regression and a line of agreement are plotted for comparison. Vertical bars represent low and high radiometric age estimates based on the analytical uncertainty of ^{210}Pb and ^{226}Ra measurements. Horizontal bars represent the range of annulus-derived ages for age groups. The upper limit of the radiometric age estimate for the oldest female is undefined because the analytical uncertainty of ^{210}Pb:^{226}Ra exceeds 1.0.

Discussion

In four cases, the radiometric age range encompassed the annulus-derived age estimate. The oldest female tarpon had an estimate (55 yr) that was lower than the radiometric age by 23 yr. The radiometric age range, however, encompassed the annulus-derived age by 4 years. This may indicate that the annulus-derived age estimate was correct in this case. The radiometric age validation, by itself, confirms the longevity of female Atlantic tarpon to at least 50.6 yr, but it may indicate that age can meet or exceed 78.0 yr (Table 3). Similarly, the longevity of male Atlantic tarpon was confirmed to at least 31.8 yr, but may exceed 41.0 yr. When the CV for annulus-derived age was included in the range of age estimates, some additional samples encompassed the radiometric age estimates, but most deviant ages could not be explained by this variation.

The wide dispersion of residuals and the low coefficient of determination indicated that there were differences between radiometric age and annulus-derived age that could be explained in the interpretation of otolith growth zones (Fig. 2). According to Crabtree et al. (1995), aging otoliths of the Atlantic tarpon was confusing and many were not readable. Although Crabtree et al. (1995) validated the annual periodicity of growth zone formation for 12 out of 18 young fish up to an age of 9 yr in captivity, the pattern of growth zone formation in older, wild fish may not be annual. Because the tarpon is a migratory fish that inhabits inshore and estuarine waters of varying salinity and temperature and spawns offshore (Zale and Merrifield, 1989; Crabtree et al., 1992), there is a high potential for irregular growth zone formation from the stresses of extreme changes in habitat (Pannella, 1980; Campana, 1983). Subannual growth zones may explain annulus-

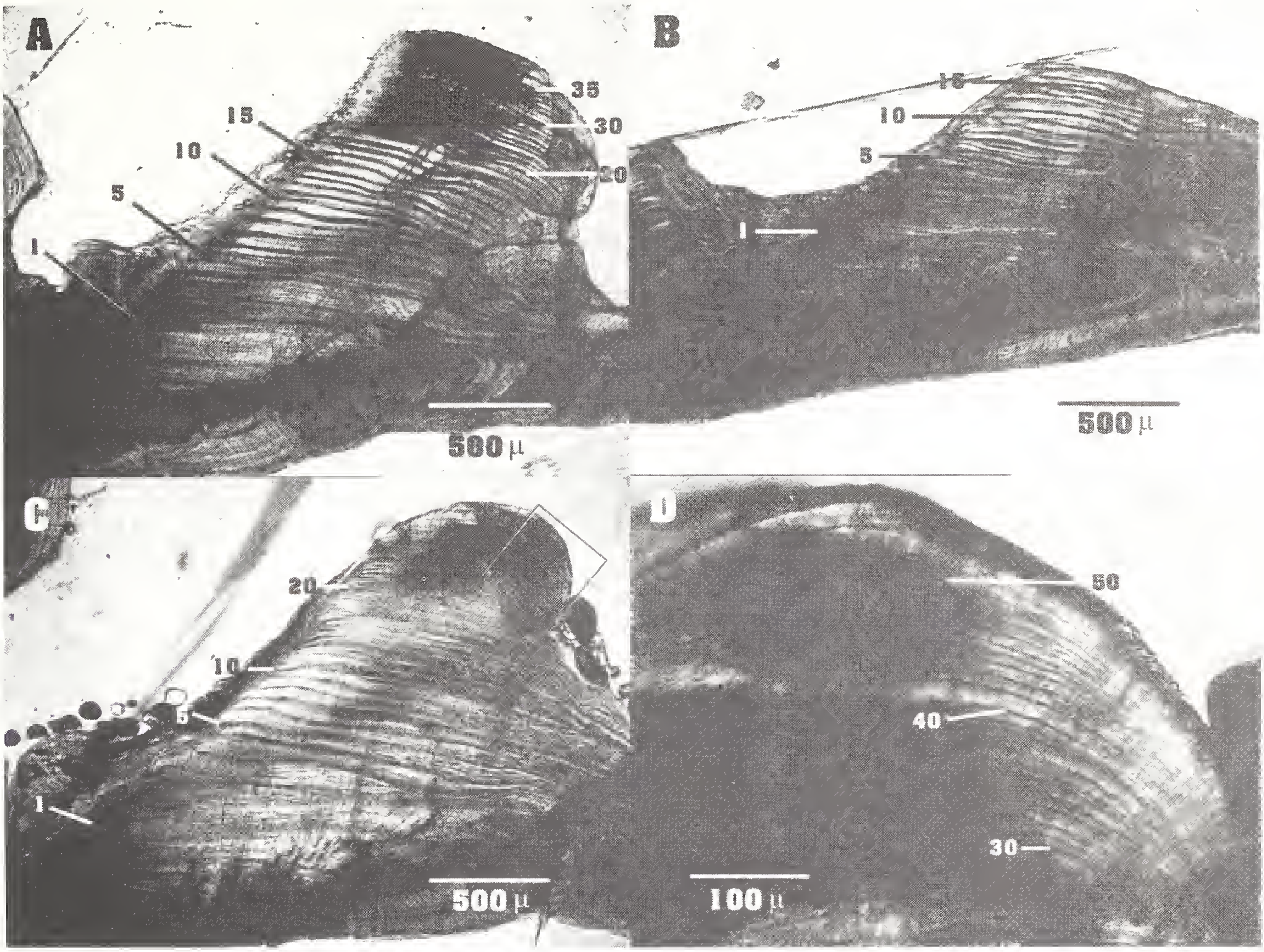

Figure 3

Transverse otolith sections from three Atlantic tarpon (*Megalops atlanticus*) illustrating the inherent difficulty in counting annuli. Annulus-derived ages were estimated by counting visible growth increments for each section shown (Crabtree et al. 1995). One sagitta was used for counting annuli and the other for radiometric aging. (**A**) An otolith section for which the number of growth increments clearly exceeds the radiometric age estimate. The annulus-derived age estimate was 37 yr and the radiometric age estimate was 24.5 yr. (**B**) An otolith section with fewer growth increments than the radiometric age estimate, but which could have had a higher annulus-derived age estimate if the reader had taken a more aggressive approach. The annulus-derived age estimate was 17 yr and the radiometric age estimate was 28.0 yr. (**C**) An otolith section with a well-developed sulcal region and numerous growth increments that rapidly become compressed toward the margin at the lower left. (**D**) Close up of the region in the same section (C) where growth increments are compressed and narrowly spaced. Note that growth increments in the region are partially obscured by what appears to be a proteinaceous inclusion. The annulus-derived age estimate was 55 yr and the radiometric age estimate was 78.0 yr.

derived age estimates that were higher than radiometric age. Poorly defined annual growth zones, or growth zones that become too small or compressed to be quantified, may explain annulus-derived ages that were lower than radiometric ages (Fig. 3). An additional consideration was that the age groups were composed of otoliths pooled together on the basis of annulus-derived age. This means that the radiometric age determined for these groups was the average age of the otoliths in the sample. Hence, a mixture of otoliths of differing ages would create an unaccountable error in the radiometric age of the age groups. This variability, however, was minimized by using otoliths of similar weight.

Von Bertalanffy growth functions fitted to the radiometric ages had growth parameters that were similar to the annulus-derived growth parameters (Crabtree et al., 1995). This determination, however, is subjective because 1) there were a low number of radiometric samples, 2) the confidence intervals were large, and 3) three out of seven male samples and three out of eight female sam-

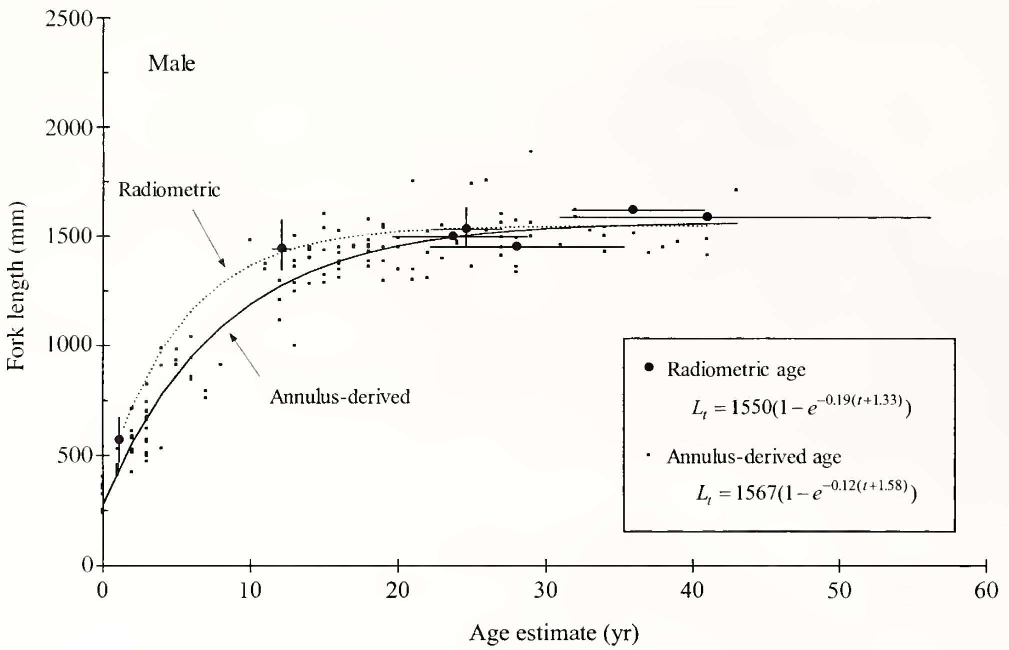

Figure 4

Von Bertalanffy growth curves for male Atlantic tarpon (*Megalops atlanticus*) with radiometric age (yr) and fork length (mm; average fork length for age groups) and with annulus-derived age (yr) and fork length. Vertical bars represent fork length range for age groups. Horizontal bars represent low and high radiometric age estimates.

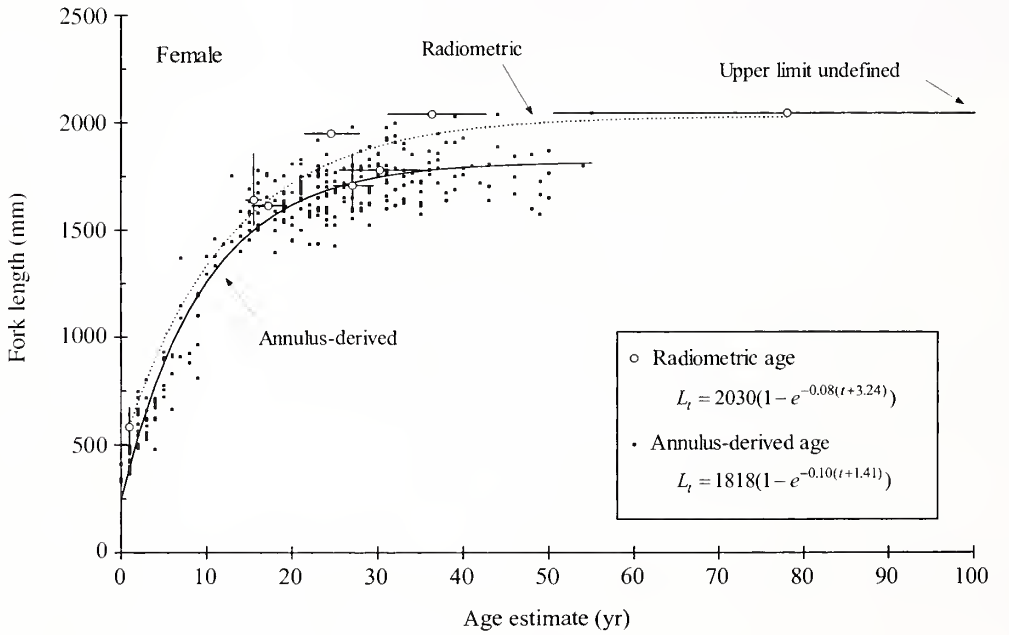

Figure 5

Von Bertalanffy growth curves for female Atlantic tarpon (*Megalops atlanticus*) with radiometric age (yr) and fork length (mm; average fork length for age groups) and with annulus-derived age (yr) and fork length. Vertical bars represent fork length range for age-groups. Horizontal bars represent low and high radiometric age estimates.

ples were an average age and average fork length for each age group. The growth coefficient for male radiometric ages (k=0.19 ±0.12) was similar to the annulus-derived estimate (k=0.12) according to the margin of error and was driven largely by one middle-age sample (Fig. 4). The growth coefficient for female radiometric ages (k=0.08 ±0.04) was also similar to the annulus-derived estimate (k=0.10). The male asymptotic length for radiometric ages (L_∞=1550 ±83 mm FL) encompassed the result from annulus counts (L_∞=1567 mm FL). For females, the asymptotic length was greater (L_∞=2030 ±227 mm FL) but still encompassed the result from annulus counts (L_∞=1818 mm FL). The closeness of the radiometric estimate of L_∞ to the maximum size was probably driven by the exceptionally large tarpon used in our study (Fig. 5).

The large size of the 2-year-old otolith core, coupled with the relatively high ^{226}Ra activity and high sensitivity of the TIMS technique, permitted age determination of smaller samples than previously possible (Kastelle et al., 1994; Fenton and Short, 1995). In a recent radiometric aging study of the blue grenadier (*Macruronus novaezelandiae*), six pooled otolith cores were needed to attain a sample weight of approximately 1 g with measurable ^{226}Ra activity (Fenton and Short, 1995). Although the ^{226}Ra activity for the samples in that study were a factor of 10 lower than the ^{226}Ra activities observed in our study, the technique used was less sensitive than TIMS and resulted in relatively high analytical uncertainties (12–21%). In a recent study of sablefish (*Anoplopoma fimbria*), where radium activities were similar to the activities observed in our study, the margin of error was relatively low (~4%), but otolith cores still needed to be pooled to attain approximately 1 g (Kastelle et al., 1994). The use of TIMS to determine ^{226}Ra in our study made it possible to age age-groups that were approximately one third of a gram and individual otolith cores that were less than 0.1 g with analytical uncertainties typically less than 2%. Because of this advance, the greatest contribution to the aging error was no longer from ^{226}Ra determination, but from the determination of ^{210}Pb by means of α-spectrometry (Andrews et al., 1999b). In our study, the determination of ^{210}Pb activity contributed from 77% to 90% of the aging error.

The radiometric aging technique is well supported as a valid aging tool in numerous fish aging studies (Bergstad, 1995; Burton et al., 1999). In our study, ^{226}Ra activities varied by approximately an order of magnitude, but the ^{210}Pb activities never exceeded the activity of ^{226}Ra. Therefore, it is highly unlikely that ^{210}Pb was not the result of ingrowth from ^{226}Ra incorporated during otolith formation. By performing a complete analysis on individual otoliths, we found that we could use radiometric age estimates with more confidence than estimates derived from pooled otolith samples. More recent successes with this technique (Andrews et al., 1999a) suggest that it is a reliable means to obtaining accurate age estimates. Radiometric age determinations in our study, therefore, indicate that the interpretation of growth zones in Atlantic tarpon otoliths can be difficult, and in some cases can be inaccurate. In addition, our study provides conclusive evidence that the longevity of the Atlantic tarpon is greater than 50 years.

Conclusions

Accurate age determination of heavily harvested fish species is critical to the formulation of responsible management strategies. The typical growth-zone validation techniques have limited applicability to long-lived fishes (Mace et al., 1990; McFarlane and Beamish 1995). Underestimated longevity and overfishing were factors that led to the decline of the Pacific ocean perch (*Sebastes alutus*) and the orange roughy (*Hoplostethus atlanticus*; Beamish, 1979; Smith et al., 1995). Because of the highly variable growth zone patterns (Fig. 3) and irregular life history of the Atlantic tarpon, conventional aging methods appear to be problematic for this species. The improved radiometric aging technique used in our study incorporated a growth-zone independent chronometer that, when judiciously applied, will enable accurate age determination of many threatened species. At a time when many fish species are suffering from fishing pressure and changing oceanographic conditions, new methods need to be applied to help ascertain appropriate management strategies. The radiometric aging technique, which has been successfully applied to the sablefish, whose longevity has been validated with other techniques (Kastelle et al., 1994, Beamish and McFarlane 2000), promises to be a valuable tool for aging species with unknown or difficult-to-interpret otolith growth patterns.

Acknowledgments

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Effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch

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Abstract—Experiments were conducted to determine the effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch. Bioassays were conducted on five economically important bycatch species: spotted seatrout (*Cynoscion nebulosus*); red drum (*Sciaenops ocellatus*); Atlantic croaker (*Micropogonias undulatus*); southern flounder (*Paralichthyes lethostigma*); and blue crab (*Callinectes sapidus*). Red drum were most affected by hypersalinity, requiring 17 minutes exposure to a 70‰ salt water solution to kill 50% of the test specimens within 48 hours. For samples collected from commercial boats and Texas Parks and Wildlife (TPW) trawl samples, we found that neither initial nor final percent survival was significantly different for bycatch removed with or without the aid of a salt-box. Bycatch mortality was high regardless of the method used to separate bycatch from the target catch. At the conclusion of catch separation, bycatch survival averaged 76% (±22%) for commercial samples and 48% (±40%) for TPW trawl samples separated with salt-boxes. Survival at the conclusion of catch separation without a salt-box averaged 56% (±35%) for commercial samples and 43% (±39%) for TPW trawl samples. Bycatch survival 21–27 h after catch separation averaged 13% (±6%) for commercial samples and 5% (±9%) for TPW trawl samples separated with salt-boxes and 34% (±29%) for commercial samples and 10% (±19%) in TPW trawl samples separated without a salt-box. Mortality rates (M) for bycatch separated with a salt-box averaged 0.08 (±0.03) for commercial samples and 0.10 (±0.04) for TPW trawl samples. For bycatch separated without the salt-box, M averaged 0.48 (±1.23) for commercial samples and 0.10 (±0.05) for TPW trawl samples. Results of an exploratory analysis with stepwise multiple regression suggested that final percent survival of bycatch was most affected by trawling time. The salt-box had little or no effect on bycatch survival; therefore, regulating the use of salt-boxes in shrimp trawling operations is not necessary.

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Shrimp (*Penaeus* spp.) are the most important commercial seafood product in Texas, accounting for more than 80% by weight and 93% of the value of all Texas commercial fisheries landings (Robinson et al., 1996). Texas shrimp landings from 1986 through 1995 were about 32,000–44,000 metric tons (t) annually. About 6400–8000 t of the annual catch during the 10-year period came from Texas bays. Texas bay shrimpers are reported to catch 2.4–6.8 kg of nontarget species (bycatch) for each kg of shrimp landed (Fuls and McEachron, 1998). Removal of the bycatch is time consuming and thus costly to fishermen. To reduce the time spent removing bycatch, Texas bay shrimpers frequently use salt-boxes to assist in rapid separation of bycatch from shrimp (Bumguardner and Colura, 1997). Salt-boxes are onboard tanks that hold a hypersaline solution of seawater and food-grade salt. The catch is placed in the solution, where most fish species float and shrimp sink. Floating bycatch is skimmed from the surface and discarded. Shrimp are then dipped from the tank and any remaining bycatch removed and discarded. Although use of salt-boxes is known to fisheries managers, the effect of its use on the survival of discarded organisms is unknown.

The objectives of our study were to assess the effects of the use of the salt-box on bycatch survival. This was accomplished by first conducting bioassays to determine the exposure time to hypersaline conditions that would affect survival of five economically important species known to occur as a part of the bycatch (Fuls and McEachron, 1998). The combined effects of trawling and exposure to hypersaline conditions in the salt-box were evaluated by comparing survival and mortality rates of bycatch (all species) and three major components of the bycatch (Atlantic croaker, *Micropogonias undulatus,* sand seatrout, *Cynoscion arenarius*, and spot, *Leiostomus xanthurus*) separated with and without the aid of a salt-box. This comparison was made for a series of samples collected from Texas bay shrimp fishermen and a series of samples collected during control experiments conducted by Texas Parks and Wildlife (TPW) personnel using experimental trawls. The TPW control experiments were conducted to remove some of the variation caused by the differing fishing methods used by each bay shrimper (i.e. different lengths of time the trawl was fished).

Materials and methods

Bioassays

Bioassays were conducted on spotted seatrout (*Cynoscion nebulosus*), red drum (*Sciaenops ocellatus*), Atlantic croaker, southern flounder (*Paralichthys lethostigma*), and blue crab (*Callinectes sapidus*). The species were selected because of their importance to Texas' recreational or commercial fisheries, or both (Weixelman et al., 1992; Robinson et al., 1996) and because they are known to occur as a portion of the bycatch (Fuls and McEachron, 1998). Red drums and blue crabs were col-

Table 1

Exposure times, salinity, temperature, and number of individuals per replicate used in high-salinity exposure bioassays. Three replicates were used at each exposure time.

Species	Exposure times (min)	Salinity (‰)	Temperature (°C)	Bay water salinity (‰)	Fish/ replicate
Red drum	4, 8, 16, 32, 64, 128	70	24	19	10
Atlantic croaker	4, 8, 16, 32, 64, 128	70	23	16	5
Spotted seatrout	4, 8, 16, 32, 64[1]	69	26	24	5
Southern flounder	4, 8, 16, 32, 64, 128	71	27	18	5
Blue crab	4, 8, 16, 32, 64, 128	70	26	24	5

[1] Specimens did not survive to 128 min of exposure.

lected from TPW hatchery ponds; Atlantic croaker and southern flounder were collected from Matagorda Bay with a trawl; spotted seatrout were collected form East Matagorda Bay with a bag seine. Fish were held 7–10 days in 1850-L tanks at ambient bay salinities and temperatures and fed frozen brine shrimp or chopped shrimp (or both) three times daily. Blue crabs were placed in holding tanks for one day prior to bioassay initiation. Juvenile blue crabs molt at frequent intervals and because of their aggressive nature, any soft crabs in a common tank are almost immediately cannibalized. Therefore, blue crab trials were held before substantial mortality occurred in holding tanks and without an initial acclimation period.

Bioassay trials were conducted at salinities of 70‰ (±1‰) and temperatures from 23 to 27°C. A salinity of 70‰ was selected on the basis of salinities observed in salt-boxes on commercial shrimping vessels (Bumguardner and Colura, 1997). Trials were conducted over a geometric sequence of times up to 128 minutes with three replicates for each time (Table 1). Either 5 or 10 individuals were exposed for each replicate, depending on size and availability. Test animals were placed in 4-L all-glass aquaria that had been washed with acid and alcohol and filled with about 3-L of 70‰ salt water. Air was continuously bubbled through the water from a 1-mL disposable pipette attached to an air blower. High-salinity water was obtained by adding salt (Mortons Ship n Shore®) purchased from a fishing supply store to water taken from Matagorda Bay. Salinity levels of bay water for each trial are presented in Table 1. Animals were removed from test aquaria at the end of the specified exposure time, high-salinity water was discarded, the aquarium was refilled with bay water, and animals were replaced in the aquaria. So that control animals were treated similarly, they were placed in the aquaria containing bay water and held for 60 minutes, after which these animals were removed, the bay water replaced with new bay water, and the control animals returned to the aquaria. The number of live individuals was recorded for each replicate immediately after high-salinity exposure, 24 hours after exposure, and 48 hours after exposure. For each species, probit analysis was conducted on untransformed data to determine the time at which 50% of the test animals exposed to the test salinity died (LE_{50}) (Sokal and Rohlf, 1981). A no-observed-effect exposure (NOEE) (Rand and Petrocelli, 1985) for each species was calculated by using a multiple goodness-of-fit comparison of log-likelihood ratio *G* values (Sokal and Rohlf,1981).

Effect of salt-box use on bycatch survival: samples from the bay shrimp industry

Thirty samples of bycatch were collected from May through October 1995 from bay shrimpers working in Matagorda, San Antonio, and Aransas bays. Fifteen samples were separated with a salt-box (SB) and the remaining 15 samples were separated without the aid of a salt-box (NSB). For each sample the following data were collected:

1 Length of time that trawl was fished;
2 Time that catch was removed from water;
3 Time that separation procedure began;
4 Time that bycatch was returned to water;
5 Bay temperature;
6 Bay salinity; and
7 Salinity in salt-box.

Total catch weight was not measured because bay shrimpers do not weigh individual catches and we did not want to change their shrimping practices more than necessary to collect the samples. To collect bycatch samples, an individual boarded the boat and waited until the bycatch was discarded. A 1–2 kg sample was randomly removed from the bycatch with a dip net or plastic scoop and placed into a 60-cm diameter × 60-cm deep holding pen. Pens were constructed from a bag of 15-mm stretch-mesh knotless nylon net secured to a life ring. The opening in the life ring served as the opening of the pen. After the sample was placed in the pen, the opening was covered with 6-mm stretch-mesh netting. The pen was then placed overboard where TPW personnel in another boat recovered it. The samples, when brought aboard the TPW boat, were immediately placed in separate 89-L transport tanks (ice chests fitted with plastic tubing and commercial aquarium air stones, through which compressed oxygen was supplied to the tanks). Dead organisms were removed from samples immediately after placement in the transport tanks.

All moribund specimens were classified as dead. Samples were transported to the Perry R. Bass Marine Fisheries Research Station (PRB) located near Palacios, Texas, where they were transferred to separate 60-cm diameter × 60-cm deep holding pens placed in an aerated 1500-L holding tank. Water in the holding tank was maintained within 2°C and 2‰ salinity of the bay temperature and salinity from which the samples were taken. Samples were checked at least hourly during transport to PRB. After having been placed in the holding tank, organisms were checked at least every three hours, except from 2300 to 0700 hours, when they were checked once. Each time samples were checked, beginning when the samples were placed in transport tanks, all dead organisms were removed, labeled to identify sample, their time and date of collection were recorded, and the organisms were preserved on ice for later examination. At approximately 1200 hours on the day following collection of the sample (about 21–27 h after collection), the remaining organisms were removed, their condition identified (living or dead), and the sample identification number and time and date of collection were recorded. All organisms collected in the sample were then identified, enumerated, and their total length (TL) was measured.

The effect of salt-box use was evaluated for all bycatch species and for Atlantic croaker alone. Three measurements were used for the evaluation: percent survival of bycatch organisms when separation of catch was completed (initial survival), percent survival of bycatch organisms at completion of sample observations (final survival), and mortality rate (M) of bycatch. Mortality rate was the absolute value of the slope of the regression of the natural log of living organisms +1 as a function of time. Time was measured from the time the catch was removed from the water to completion of sample observation. Because the three measurements had unequal variances, even when data were transformed, nonparametric procedures were used to compare the two separation methods and to examine relationships. Wilcoxon signed rank test was used to determine if initial survival, final survival, M, number of specimens collected, and TL of specimens differed significantly between the two separation methods. The Spearman correlation coefficient and the probability that the coefficient was significantly greater than 0 were used to examine relationships among all variables. For the correlation analysis, the use of a salt-box was coded as 1 and non-use as 0.

Stepwise multiple regression analysis was used as an exploratory procedure to examine the effect of 1) length of time trawl was fished; 2) catch separation time (total time bycatch was onboard the vessel); 3) bay temperature at the time of collection; 4) bay salinity at time of collection; and 5) effect of use or non-use of a salt-box on initial survival, final survival, and M of bycatch. For the regression analysis, coding of the use and non-use of a salt-box was the same as that for the correlation analysis. The significance level of the independent variable to enter and stay in the model was $P \leq 0.05$. Statistical analyses were performed by using the Statistical Analysis System (SAS Institute, 1990). All statistical tests were considered significant at the $P=0.05$ level.

Effect of salt-box use on bycatch survival: control experiments

Fourteen samples were collected from Matagorda Bay between 16 May and 25 October 1995, with a 6.1-m wide trawl constructed of 3.8-cm stretched nylon multi-filament mesh (Kana et al., 1993) equipped with a 6-mm stretch-mesh liner in the codend. The trawl was towed for 15 minutes. The catch was removed from the trawl and placed on a 0.67 × 1.0 m table. The catch was allowed to sit on the table for seven minutes to simulate the time the catch sits on deck while commercial shrimpers re-initiate trawling operations. The 7-min period was selected on the basis of a preliminary survey that we conducted of Texas bay shrimp fishing practices. The catch was then divided into approximately two equal portions: one half was placed in a salt-box and the bycatch was removed by skimming floating organisms from the surface; the remaining half of the catch remained on the table and shrimp were removed by hand, leaving the bycatch on the table. The salt-box was a 185-L fiberglass tank, half filled with bay water to which was added about 7.5 kg of food-grade salt. All bycatch removed from the salt-box was immediately placed in a separate 89-L transport tank filled with bay water and supplied with compressed oxygen. In the hand separation process, shrimp were removed and the bycatch remaining on the table was placed in a separate 89-L transport tank filled with bay water and supplied with compressed oxygen. Dead organisms were removed from samples immediately after they were placed in transport tanks. Samples were then transported to the PRB where they were treated in the same manner as previously described for samples collected from commercial shrimpers. The effect of the salt-box on the bycatch (all species), and on Atlantic croaker, spot, and sand seatrout (each species separately) was evaluated. Analyses were identical to those used to analyze samples collected from bay shrimpers.

Results

Bioassays

Probit analysis indicated LE_{50} for the five species ranged from a low of 17 min for red drum to 67 min for blue crab (Table 2). Southern flounder was the most tolerant of the fishes with an LE_{50} of 58 min. Comparison of log-likelihood ratio G values indicated that NOEE ranged eight min for Atlantic croaker and red drum to 64 min for blue crab. Southern flounder had the greatest NOEE (32 min) of the four fishes tested.

Effect of salt-box use on bycatch survival: samples from the bay shrimp industry

All commercial fishermen resumed fishing operations before beginning the catch separation procedure. Therefore, for descriptive purposes, catch separation time can be divided into two periods: 1) time the catch remains on deck or table while fishing is resumed and 2) time the catch is

Table 2

Results of high salinity bioassays and mean total length (TL) of experimental animals. LE_{50} = time required for 50% of experimental animals to die, NOEE = no-observed-effect from exposure time.

Species	n	LE_{50} (min)	NOEE (min)	Mean TL (mm)
Atlantic croaker	210	19	8	65 ±11.7
Red drum	105	17	8	28 ±2.7
Spotted seatrout	90	35	16	72 ±15.5
Southern flounder	105	58	32	131 ±23
Blue crab	105	67	64	45 ±4.8[1]

[1] Carapace width for blue crabs = TL.

Table 3

Mean (±SD) trawling time (h), catch separation time (min), bay salinity (‰), salinity in the salt-box (‰), and bay temperature (°C) for bycatch samples collected from the commercial fishery and separated with (n=15), or without (n=15), the aid of a salt-box and results of Wilcoxon signed rank tests comparing the means.

Variable	Salt-box	No salt-box	Z	P
Trawling time	1.5 ±0.62	1.2 ±0.79	0.634	0.526
Separation time	9.9 ±3.1	16.5 ±21.5	–0.605	0.550
Bay salinity	21.0 ±4.7	22.3 ±2.7	–1.045	0.296
Salt-box salinity	77.0 ±6.4			
Bay temperature	26.7 ±0.7	28.7 ±1.8	–3.887	<0.001

sorted. The mean time that catch remained on deck while fishing resumed was 11.7 min (±18.9 min) for fishermen not using a salt-box and 7.8 min (±4.2 min) for fishermen using a salt-box. Time the catch remained on the deck was statistically similar for the two methods (Z=0.6251, P=0.9502). The catch separation procedure with a salt-box began when fishermen used a large scoop to transfer the catch from the boat deck to the salt-box. Fishermen not using a salt-box spread the catch on the boat deck or a table and began removing shrimp. Once the catch separation procedure began, fishermen using a salt-box required 1.7 min (±1.4 min) to separate the catch, whereas fishermen not using a salt-box required 7.0 min (±12.0 min) to separate the catch; mean time required was statistically similar between the methods (Z=–1.44571, P=0.1483). Time required to separate the catch once the procedure began appeared to depend upon size of the catch, but we had only the captain's estimate of the size of the catch. Trawling time, catch separation time (total time bycatch was out of the water), and bay salinity at the time samples were collected did not differ significantly for samples collected by each method (Table 3). Mean bay temperature at the time of collection differed significantly between samples collected with the salt-boxes and those collected with no salt-box. Bay temperature averaged 28.7° (±1.8°)C when NSB samples were collected and 26.7° (±0.7°)C when SB samples were collected.

Thirty-three species (30 fishes and 3 invertebrates) were collected (Table 4). Atlantic croaker (n=536) was the most common organism and was present in 27 of the 30 samples. Other fishes frequently observed were spot (n=278), sand seatrout (n=90), Gulf menhaden (*Brevoortia patronus*, n=198), and bay anchovy (*Anchoa mitchilli*, n=45). The most frequently observed invertebrates were brown shrimp (*Penaeus aztecus*, n=46) and blue crab (n=34). Remaining species generally averaged <1 per sample. Some differences were observed in the frequency and length of some of the bycatch species that composed the NSB and SB samples. However, differences were generally small and were probably biologically insignificant. Atlantic croaker, bay anchovy, hardhead catfish (*Arius felis*), and brown shrimp were found in significantly greater numbers in SB samples than in NSB samples (Table 5). Bay whiff (*Citharichthys spilopterus*), bluntnose jack (*Hemicaranx amblyrhynchus*), Atlantic spadefish (*Chaetodipterus faber*), and inshore lizardfish (*Synodus foetens*) were found in significantly greater numbers in NSB samples than in SB samples, although they were rare in NSB samples. Mean number of specimens collected for all other species were similar in both sample types. Mean length of Atlantic croaker, Gulf menhaden, spot, sand seatrout, and pinfish (*Lagodon rhomboides*) were significantly greater in NSB samples (Table 5). Blue crabs collected in SB samples had a significantly greater mean carapace width than those collected in NSB samples. Mean lengths of all other species were similar in both sample types.

In general, all three measures of salt-box effect on bycatch were highly variable (Table 6). Mean (±SD) initial

Table 4

Species collected from bycatch of Texas bay shrimpers and in Texas Parks and Wildlife (TPW) samples. X indicates that the species was collected.

Common name	Species	Bay shrimpers	TPW
Vertebrates			
Atlantic bumper	*Chloroscombrus chrysurus*	X	
Atlantic croaker	*Micropogonias undulatus*	X	X
Atlantic cutlassfish	*Trichiurus lepturus*	X	X
Atlantic midshipman	*Porichthys plectrodon*	X	X
Atlantic spadefish	*Chaetodipterus faber*	X	X
Atlantic stingray	*Dasyatis sabina*	X	
Atlantic threadfih	*Polydactylus octonemus*		X
bay anchovy	*Anchoa mitchilli*	X	X
bay whiff	*Citharichthys spilopterus*	X	X
bighead searobin	*Prionotus tribulus*	X	X
blackcheek tonguefish	*Symphurus plagiusa*	X	X
bluefish	*Pomatomus saltatrix*	X	
bluntnose jack	*Hemicaranx amblyrhynchus*	X	X
gafftopsail catfish	*Bagre marinus*	X	X
gulf butterfish	*Peprilus burti*		X
gulf menhaden	*Brevoortia patronus*	X	X
hardhead catfish	*Arius felis*	X	X
harvestfish	*Perprilus alepidotus*	X	X
hogchoker	*Trinectes maculatus*	X	X
inshore lizardfish	*Synodus foetens*	X	X
least puffer	*Sphoeroides parvus*	X	X
lookdown	*Selene vomer*	X	
pinfish	*Lagodon rhomboides*	X	X
sand seatrout	*Cynoscion arenarius*	X	X
silver jenny	*Eucinostomus gula*		X
silver perch	*Bairdiella chrysoura*	X	X
skipjack herring	*Alosa chrysochloris*	X	
southern flounder	*Paralichthys lethostigma*	X	X
southern kingfish	*Menticirrhus americanus*	X	
spot	*Leiostomus xanthurus*	X	X
spotted seatrout	*Cynoscion nebulosus*	X	
star drum	*Stellifer lanceolatus*	X	
threadfin shad	*Dorosoma petenense*	X	
Invertebrates			
Atlantic brief squid	*Lolliguncula brevis*	X	X
blue crab	*Callinectes sapidus*	X	X
brown shrimp	*Penaeus aztecus*	X	X
white shrimp	*Penaeus setiferus*		X

survival rates were 76.1% (±21.7%) and 55.5% (±35.1%), respectively for SB and NSB samples. Final survival rates were 12.9% (±6.0%) for SB samples and 34.5% (±28.9%) for NSB samples. Means of initial and final survival were not significantly different. Average (±SD) M of organisms separated by use of a salt-box (0.08 [±0.03]) were significantly lower than M of bycatch separated without the aid of a salt-box (0.48 [±1.23]).

For individual species, comparison of the effect of use and non-use of a salt-box on M, and on initial and final survival variables could only be determined for Atlantic croaker. Other species were either found infrequently in individual samples, or in insufficient numbers within the sample type. Estimates of initial and final survival for Atlantic croaker were obtained from 19 samples (6 NSB samples and 13 SB samples). Estimates of M were obtained from 16 samples (4 NSB samples and 12 SB samples). Samples not used had too few Atlantic croaker (i.e. only one or two specimens in the sample or no deaths) to make estimates of percent survival or M. Means of the three estimates used to compare the two separation techniques were highly variable for Atlantic croaker (Table 6). Means

Table 5

Species which differed significantly in either mean numbers or total length (mm) from comparative samples collected from the commercial fishery and separated with or without the aid of a salt-box, and results of Wilcoxon signed rank tests used to compare means.

	Salt-box		No salt-box			
Species	Mean ±SD	n	Mean ±SD	n	Z	P
Number of specimens						
bay anchovy	2.7 ±4.9	15	0.3 ±0.6	15	3.159	0.002
Atlantic croaker	28.8 ±14.5	15	6.9 ±5.0	15	4.030	<0.001
hardhead catfish	2.5 ±3.3	15	0.7 ±1.4	15	2.150	0.032
bay whiff	0.1 ±0.3	15	1.3 ±1.4	15	–3.097	0.002
bluntnose jack	0	15	1.3 ±1.7	15	3.440	0.001
Atlantic spadefish	0	15	0.5 ±0.9	15	–2.366	0.018
inshore lizardfish	0	15	0.4 ±0.8	15	–2.073	0.038
brown shrimp	2.0 ±1.8	15	1.1 ±1.8	15	3.397	0.001
Total length						
Atlantic croaker	120 ±15	419	104 ±19	93	8.129	<0.001
gulf menhaden	110 ±47	120	119 ±22	57	3.460	<0.001
spot	98 ±23	157	109 ±20	60	3.910	<0.001
sand seatrout	103 ±30	45	129 ±25	44	4.830	<0.001
pinfish	75 ±13	9	89 ±20	82	–3.348	0.001
blue crab	82 ±23	60	66 ±28	16	–2.268	0.023

Table 6

Means of bycatch (all species) and Atlantic croaker initial percent survival, final percent survival, and mortality rate (M) and results of Wilcoxon signed rank tests comparing the means. All samples were collected from Texas bay shrimpers and separated with or without the aid of a salt-box.

	Salt-box		No salt-box			
Variable	Mean ±SD	n	Mean ±SD	n	Z	P
Bycatch						
initial survival	76.1 ±21.7	15	55.5 ±35.1	15	1.763	0.078
final survival	12.9 ±6.0	15	34.5 ±28.9	15	–1.389	0.093
M	0.08 ±0.03	15	0.48 ±1.23	15	2.605	0.009
Atlantic croaker						
initial survival	74.4 ±25.7	13	81.8 ±25.1	6	0.746	0.046
final survival	28.6 ±19.9	13	62.3 ±30.7	6	2.330	0.020
M	0.23 ±0.12	12	0.26 ±0.19	4	0.364	0.716

of M were similar between separation techniques. Means of initial (81.8% [±25.1%]) and final (62.3% [±30.7%]) survival of fish separated without a salt-box were significantly greater than initial (74% [±25.7%]) and final (28.6% [±19.9%]) survival of those separated with a salt-box.

By examining Spearman correlation coefficients that were significantly greater than 0, we found a relationship between the three measurements and factors other than use or non-use of a salt-box (Table 7). Initial and final survival estimates were negatively correlated to trawling time and catch separation time, whereas M was positively correlated to the two variables. Only estimates of M (r_s=0.488) were found to be related to the use or non-use of a salt-box. Final estimates of survival and M were also associated with some bycatch species. M was positively correlated to numbers of Atlantic croaker (r_s=0.526) and sand seatrout (r_s=0.458) in the samples, whereas final survival was inversely correlated to the two species (Atlantic croaker r_s=–0.391, sand seatrout r_s=-0.564). Other species to which M and final survival were associated occurred in fewer than half the samples or averaged <1 specimen per sample. Initial survival estimates were not related to

Table 7

Spearman correlation coefficients (r_s) and probability (P) that the coefficient values were greater than 0, of variables that demonstrated relationships with mortality rate (M), initial percent survival, and final percent survival for bycatch (all species) and Atlantic croaker. Samples were collected from the catch of Texas bay shrimpers and separated with or without a salt-box. A space indicates the probability that the variables r_s value was greater then 0 was >0.05.

	M		Initial survival		Final survival	
Variable	r_s	P	r_s	P	r_s	P
Bycatch						
separation time	0.638	<0.001	–0.586	0.001	–0.749	<0.001
trawling time	0.650	<0.001	–0.414	0.023	–0.665	<0.001
bay temperature	–0.459	0.011				
bay anchovy	0.477	0.008				
Atlantic croaker	0.526	0.003			–0.391	0.033
gulf menhaden	0.445	0.014			–0.461	0.010
sand seatrout	0.458	0.011			–0.564	0.001
pinfish	–0.434	0.017				
bighead searobin	–0.368	0.046				
gafftopsail catfish					–0.398	0.029
bay whiff					0.379	0.039
Atlantic cutlassfish					–0.373	0.042
salt-box	0.488	0.006				
Atlantic croaker						
separation time	0.637	0.008	0.459	0.016		
least puffer	0.994	<0.001				
hardhead catfish					–0.592	0.001
blue crab					–0.463	0.015
brown shrimp					–0.566	0.002
harvestfish			0.401	0.038	0.400	0.038
Atlantic brief squid	–0.833	0.039				
salt-box					–0.629	0.004

Table 8

Independent variables that entered stepwise multiple regression models describing bycatch (all species), initial percent survival, final percent survival, and mortality rate (M), and Atlantic croaker M resulting from Texas bay shrimpers separating them from shrimp with or without the aid of a salt-box. Included are y-intercepts±SE, coefficients±SE of the selected independent variables, partial r^2 of each variable in parenthesis, and model r^2. A space indicates the variable did not enter the model.

		Independent variable				
Dependent variable	y-intercept	Separation time	Trawl time	Bay temperature	Salt-box	Model R^2
Bycatch						
initial survival	217.4 ±65.3	–120.5 ±21.8 (0.52)				0.52
final survival	60.0 ±6.9		–20.2 ±4.1 (0.48)		–15.5 ±5.8 (0.11)	0.59
M	2.3 ±1.2	5.1 ±0.4 (0.79)	–0.3 ±0.1 (0.03)	–0.1 ±0.04 (0.03)		0.85
Atlantic croaker						
final survival	51.2 ±10.1	61.9 ±27.0 (0.11)			–31.22 ±10.0(0.38)	0.49

any bycatch species. The initial survival (r_s=0.459) and M (r_s=0.637) of Atlantic croaker were related to catch separation time. Atlantic croaker final survival (r_s=–0.629) was negatively correlated to use of a salt-box.

Stepwise multiple regression identified four variables, which explained most of the variation associated with the three measurements (Table 8). Catch separation time (total time that bycatch was on the vessel), trawling time, and

Table 9

Species collected from samples of bycatch separated with or without the aid of a salt-box in control experiments whose mean total length (mm) differed significantly and results of Wilcoxon signed rank tests used to compare the means.

	Salt-box		No salt-box			
Species	Mean total length ±SD	n	Mean total length ±SD	n	Z	P
Bay anchovy	50 ±11	191	53 ±13	219	–2.977	<0.001
Spot	94 ±18	138	89 ±24	194	2.818	0.005
Silver perch	72 ±37	4	111 ±22	18	–2.605	0.009
Atlantic brief squid	82 ±39	53	58 ±39	63	4.177	<0.001

bay temperature explained 85% of the variation associated with estimates of bycatch M. Catch separation time was the most important of the three, explaining 79% of the variation. Catch separation time explained 52% of the variation observed in initial survival and was the only variable to meet the $P \leq 0.05$ significance level required for entry into the model. Trawling time explained 48% of the variation observed in bycatch final survival and use or non-use of a salt-box explained the remaining 11%. For the dependent variable, Atlantic croaker final survival, the use or non-use of a salt-box explained 38% of the variation explained by the model and catch separation time explained an additional 11%. The five independent variables did not meet the $P \leq 0.05$ significance level required to enter or stay in stepwise regression models for Atlantic croaker initial survival and M.

Effect of salt-box use on bycatch survival: control experiments

Twenty-eight species were collected for the control experiments: 24 fishes and 4 invertebrates (Table 4). Bay anchovy (n=3794), Atlantic croaker (n=2048), spot (n=949), sand seatrout (n=291), and Gulf menhaden (n=148) were the most numerous organisms and were observed in more than half the samples collected. Remaining fishes averaged <1 individual per sample. Brown shrimp (n=494) and Atlantic brief squid (*Lolliguncula brevis*, n=133) were the most numerous invertebrates caught. By species, no significant differences were found between numbers of individuals in SB and NSB samples. Bay anchovy, spot, silver perch (*Bairdiella chrysoura*), and Atlantic brief squid collected in NSB samples were significantly larger than those collected in SB samples, but differences for the most part were small or the organism was present in small numbers (Table 9). Remaining species were similar in size in both NSB and SB samples. Catch separation time averaged 12 min (±6 min) for SB samples and 18 min (±18 min) for NSB samples and was significantly different (Z=2.05399, P=0.04). At the time samples were collected, bay temperature averaged 26.0° (±3.1°)C, bay salinity, 18,0‰. (±7.2‰), and salt-box salinity 66‰ (±8‰).

Survival rates and mortality rates were highly variable for bycatch separated by both methods. Mean initial survival rates were 48.4% (±39.7%) and 43.6% (±39.3%), respectively for SB and NSB samples. Final survival rates were 5.4% (±8.9%) for SB samples and 9.5% (±18.7%) for NSB samples. Mortality rates averaged 0.1 (±0.04) for SB samples and 0.1 (±0.05) NSB samples. All estimates of survival and mortality were statistically similar (Table 10). By species, estimates of initial survival, final survival, and M for Atlantic croaker, spot, and sand seatrout were also highly variable and were statistically similar between the two methods of bycatch separation.

Spearman correlation coefficients significantly greater than 0 demonstrated a relationship between bycatch (all species) catch separation time and the variables initial survival (r_s=0.445) and M(r_s=0.522). (Table 11). Initial survival was found to have an inverse relationship with temperature. All three measurements of the effect of salt-box use on bycatch were associated with the frequency of occurrence of some species in the bycatch. The variable M was positively correlated to Atlantic croaker (r_s=0.765), spot (r_s=0.681), and sand seatrout (r_s=0.792) numbers. Initial survival was positively correlated to Atlantic croaker (r_s=0.630), spot(r_s=0.530), and blue crab (r_s=0.494) numbers and was inversely related to the numbers of bay anchovy (r_s=–0.795) and Gulf menhaden (r_s=–0.466). Final survival was positively correlated to blue crab (r_s=0.631) numbers and inversely related to bay anchovy numbers (r_s=–0.655). Other species to which M, and initial and final survival were related occurred in fewer than half the samples and averaged <1 organism per sample. Atlantic croaker M was related to bay temperature (r_s=0.828), bay salinity (r_s=–0.513), and to the presence of Atlantic brief squid (r_s=–0.833). Neither Atlantic croaker initial nor final survival estimates were correlated to other variables. Spot and sand seatrout M, initial survival, and final survival were not correlated to any variable.

For all species, stepwise multiple regression identified catch separation time, bay salinity, and bay temperature at the time of sample collection as significant variables which explained some of the observed variation in M and final survival (Table 12). The salt-box variable did not enter any model. Catch separation time explained 27% of the variation that was accounted for by the model for the dependent variable M, and an additional 11% was explained by bay salinity at time of sample collection. Bay temperature and salinity at the time of collection explained 34% of variation observed in the final survival model. No variable met the $P \leq 0.05$ significance level for entry into regression models

Table 10

Mean (±SD) of initial percent survival, final percent survival, and mortality rate (*M*) and results of Wilcoxon signed rank tests used to compare the means of bycatch (all species), Atlantic croaker, spot, and sand seatrout from bycatch samples collected in control experiments and separated with or without the aid of a salt-box.

	Salt-box		No salt-box			
Variable	Mean ±SD	*n*	Mean ±SD	*n*	*Z*	*P*
Bycatch						
initial survival	48.4 ±39.7	14	43.6 ±39.3	14	−0.460	0.643
final survival	5.4 ±8.9	14	9.5 ±18.7	14	−0.322	0.748
M	0.10 ±0.04	14	0.10 ±0.05	14	−0.277	0.782
Atlantic croaker						
initial survival	41.1 ±38.2	8	56.0 ±40.1	8	0.790	0.430
final survival	18.3 ±24.1	8	16.8 ±34.1	8	−0.373	0.709
M	0.41 ±0.77	8	0.82 ±1.27	8	0.105	0.916
Spot						
initial survival	70.9±30.3	12	71.7 ±27.3	12	−0.206	0.837
final survival	24.9±34.8	12	21.5 ±36.4	12	−0.695	0.519
M	1.30±2.71	12	2.07 ±3.26	12	0.799	0.424
Sand seatrout						
initial survival	85.7 ±28.8	11	72.3 ±26.7	11	−1.242	0.214
final survival	29.2 ±38.0	11	14.1 ±20.0	11	−0.774	0.439
M	3.58 ±6.31	11	4.27 ±6.38	11	0.417	0.677

Table 11

Spearman correlation coefficients (r_s) and probability (*P*) that the coefficient values were greater than 0, of variables which demonstrated relationships with bycatch (all species) mortality rate (*M*), initial percent survival and final percent survival and Atlantic croaker *M*. Samples were collected in Texas Parks and Wildlife trawls and separated from shrimp with or without the aid of a salt-box during control experiments. A space indicates the probability that the variables r_s value was greater then 0 was >0.05.

	M		Initial survival		Final survival	
Variable	r_s	*P*	r_s	*P*	r_s	*P*
Bycatch						
separation time	0.522	0.004	0.445	0.018		
Atlantic croaker	0.765	<0.001	0.630	<0.001		
spot	0.681	<0.001	0.530	0.004		
sand seatrout	0.792	<0.001				
least puffer	0.692	0.039				
bay temperature			−0.4249	0.025		
salt-box salinity			−0.680	<0.001	−0.635	0.015
bay anchovy			−0.795	<0.001	−0.655	<0.001
gulf menhaden			−0.466	0.012		
bay whiff			0.444	0.018		
brown shrimp			0.433	0.021		
brief squid			−0.588	0.001		
blue crab			0.494	0.008	0.631	<0.001
silver perch			−0.386	0.042	−0.480	0.010
bluntnose jack			−0.568	0.002		
Atlantic croaker						
bay temperature	0.829	0.042				
bay salinity	−0.514	0.028				
Atlantic brief squid	−0.833	0.039				

Table 12

Independent variables which entered stepwise multiple regression models describing bycatch (all species), Atlantic croaker, spot, and sand seatrout short-term final percent survival, or mortality rate (M) (or both) resulting from their separation from shrimp with or without the aid of a salt-box during controlled experiments. Included are y-intercepts (±SE), coefficients (±SE) of the independent variables, partial R^2 of each variable in parenthesis, and model R^2. A space indicates the variable did not enter the model.

		Independent variable			
Dependent variable	y-intercept	Separation time	Bay salinity	Bay temperature	Model R^2
Bycatch					
final survival	216.59 ±58.069		−2.71 ±0.88 (0.13)	−4.68 ±2.08 (0.21)	0.34
M	0.12 ±0.03	0.09 ±0.03 (0.27)	−0.002 ±0.001 (0.11)		0.38
Atlantic croaker					
M	−5.36 ±203	2.25 ±0.45 (0.75)		0.21 ±0.08 (0.08)	0.83
Spot					
M	−1.44 ±1.41		0.18 ±0.07 (0.20)		0.20
Sand seatrout					
final survival	213.31 ±67.36			−7.08 (0.29)	0.29
M	−39.75 ±12.90			1.61 ±0.47 (0.37)	0.37

for the dependent variable initial survival. Time required to separate catch accounted for 75% of the variation observed in Atlantic croaker M, whereas bay temperature at time of sample collection accounted for the remaining 8% variation explained by the model. Bay salinity at time of sample collection explained 20% of the variation observed in M of spot. Bay temperature at time of sample collection explained 37% and 29%, respectively, of the dependent variables M and initial survival of sand seatrout. No variable met the $P \leq 0.05$ significance level for entry into models to explain initial survival of Atlantic croaker, spot, or sand seatrout, or final survival of Atlantic croaker or spot.

Discussion

Use of a salt-box to separate bycatch, as practiced by Texas shrimpers, had little or no effect on short-term survival of bycatch. Measurements of bycatch (all species) initial and final survival were not significantly different for the bycatch separation methods in samples collected from bay shrimpers and from TPW trawls. Significant differences between SB and NSB Atlantic croaker initial and final survival of samples collected from the commercial fishery were not corroborated by the results of the TPW control experiments. Exposure time to hypersaline conditions in a salt-box is short, generally less than two min, and the no-observed-effect exposure for selected species suggests that longer exposure (at least eight min for red drum and Atlantic croaker) would probably be required to significantly affect survival of most bycatch species. For these reasons, regulation of salt-box use as currently practiced by Texas bay shrimpers is unnecessary.

The use of a salt-box had little impact on bycatch M, and initial or final survival. Salt-box use was correlated to M, for samples collected from the fishery, but not to initial or final survival. Salt-box use was not correlated to any of the three variables for bycatch in the control experiments. Atlantic croaker final survival was negatively correlated to the presence of a saltbox in samples collected from the fishery but the correlation was not present in the control experiments. The salt-box variable entered the final survival regression models for combined catch and Atlantic croaker from the fishery but entered no other regression models.

Environmental factors at the time of sample collection played a role in determining survival of bycatch, although not as great as other variables associated with bycatch separation. Both bay temperature and salinity at the time of sample collection affected survival and M variables in the control experiments as demonstrated by the correlation and regression analyses. Bay temperature also explained a small portion of the variation (3%) associated with M in trials conducted on samples collected from the fishery. For individual species, bay temperature and salinity, either individually or combined, explained part of the variation associated with the regression models for M of Atlantic croaker, spot, and sand seatrout, and for final survival of sand seatrout. The effect of temperature on M in samples taken from the fishery and survival and M in control experiments suggest there may be a seasonal effect on survival of bycatch. Failure to observe a greater effect in trials with the fishery samples probably is due to the effect of other factors associated with the fishery, such as long trawl times and catch separation times that mask the effect of environmental factors.

Catch separation time was one of the most important variables associated with bycatch survival. It was correlated to M, initial survival, and final survival for samples collected from the fishery and to M and initial survival in the control experiment. The variable entered multiple regression models for the variables M and initial survival of bycatch in experiments conducted with samples collected from the fishery. It also entered multiple regression models for the dependent variable M of bycatch and Atlantic croaker in the control experiments. The use of the salt-box significantly reduced catch separation time in control experiments, suggesting that the use of the salt-box could possibly reduce M by speeding catch separation time. The importance of reducing catch separation time has been corroborated by Ross and Hokenson (1997) who reported a positive correlation between mortality and on-deck sorting time for three species of bycatch (American plaice, *Hippoglossoides platessoides*; witch flounder, *Glyptocephalus cynoglossus*; and pollock, *Pollachius virens*) associated with the Gulf of Maine northern shrimp (*Pandalus borealis*) fishery.

Trawling time was also an important variable affecting M and survival. It was correlated to M, initial survival, and final survival of bycatch and entered the final survival regression model for bycatch from the fishery. This variable may affect survival in several ways. Longer trawl times in general result in larger catches. This would presumably increase catch separation time thus contributing to increased bycatch mortality. Long trawl times would also increase the time bycatch is in the net, thus increasing the chance of fatal injuries.

In the absence of a satisfactory method to reduce the catch of nontarget species, survival of bycatch could probably be improved by limiting the time a trawl is fished and by requiring the catch to be separated prior to resumption of fishing. These variables were the only ones found in our study that had significant impact on bycatch survival and that could easily be altered to improve survival. Enforcement of either of these proposals, however, would be difficult.

Use of percent survival estimates at the conclusion of the observation periods in our study to predict long-term survival of bycatch released into the bay is not recommended. Stress in fishes is characterized by hypersecretion of epinephrine, norepinephrine, and corticosteroids. These hormones induce secondary effects resulting in changes in metabolism, osmoregulation, and the immune systems (Mazeaud et al., 1977) that last for several days and can result in increased susceptibility to disease (Wedemeyer and McLeay, 1981). Furthermore, stress can lead to behavioral changes (Wedemeyer, 1974) that might make the fish more susceptible to predation. Specimens can be maintained under identical conditions (in the laboratory or field) to compare survival and mortality rates of individuals treated differently at capture. However, it would be speculative to project survival estimates of specimens maintained in any form of captivity to those released into the wild because it is impossible to account for predation and possibly disease.

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Trace metals in four structures of fish and their use for estimates of stock structure

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Abstract—Trace elements in calcified tissues have been suggested as one of the most powerful means for stock discrimination yet developed. The structure of choice for determining elemental composition or fingerprints is the otolith, although other structures also incorporate trace elements into their matrix. The aim of this study was to compare the elemental fingerprints of four structures (otoliths, scales, eye lenses, and spines) of a territorial reef fish to determine whether there were correlations between otoliths and each of the other structures. Elemental fingerprints of juvenile (<3 years of age) and adult fish (which may reach a maximum age of 37 years) were also compared for each structure to determine whether there may be differences between size classes of fish. All structures were analyzed by solution-based inductively coupled plasma-mass spectrometry (ICP-MS). Otoliths, scales, spines, and eye lenses differed in composition. Calcium dominated otoliths, scales, and spines but was not detected in eye lenses. Some elements, for example barium, showed significant correlations between the otolith data and that of scales and spines of both juvenile and adult fish. A multivariate test of matrix correspondence (Mantel's test) detected significant relationships between the otolith data and the data matrices for each of scales and spines of both juvenile and adult fish. Significant relationships between the otolith data and the eye lens data were detected only for juvenile fish. Significant differences were also found between juvenile and adult fish for all structures. The BIOENV multivariate analyses showed that the highest rank correlation was found between the otolith data and the scale or spine data for both juvenile and adult fish. These data suggest that the use of scales and spines may provide a nonlethal alternative to the use of otoliths for future stock discrimination studies.

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Studies of the population dynamics of marine fishes depend on the ability to distinguish separate "stocks" that may make up the total population of a single fish species. Although much of fisheries management assumes that a single population is being monitored, measures of growth, natural mortality, recruitment, and reproduction are often made for the total population because data on movement or stock separation or fidelity (or the combination of all three) are inadequate to allow separation of stocks. A variety of methods exist for identifying stocks (e.g. population parameters, capture-mark-recapture studies, physiological and behavioral characters, morphometric and meristic characters, calcareous characters, cytogenic characters, and biochemical characters; Ihssen et al., 1981), but few methods can provide a reliable measure of stock identity. Genetic techniques are frequently used but fail to differentiate stocks because a small amount of larval or adult mixing among populations makes differences undetectable (Hartl and Clark, 1989). Recently, many studies have focused on the use of "elemental fingerprints" (*sensu* Campana et al., 1994), or the elemental composition of the otoliths, as a measure of stock identity.

Initial microchemistry studies tended to focus on diadromous species and distinguished between freshwater and marine life history phases of fish (e.g. Bagenal et al., 1973; Belanger et al., 1987; Kalish, 1990; Coutant and Chen, 1993; Rieman et al., 1994). The electron microprobe was widely used for such studies but because of the "typical" concentrations of Ca, Na, Sr, K, S, and Cl found in otoliths, electron probes were effectively limited to detecting these six elements. Recently, there have been dramatic advances in analytical techniques and studies now indicate that other elements also exist in otoliths (e.g. Edmonds et al., 1989, 1991, 1992; Sie and Thresher, 1992), many of which are thought to be reflective of the environment (Fowler et al., 1995). Stock discrimination capabilities have improved with the sensitivity of recent instrumentation and the inductively coupled plasma-mass spectrometer (ICP-MS) has now become the instrument of choice for simultaneously quantifying the concentration of multiple elements and isotopes (Houk, 1986; Date, 1991).

ICP-MS has recently been used for assays of various fish tissues including otoliths (e.g. Edmonds et al., 1991; Campana and Gagne, 1995; Campana et al., 1994, 1995; Dove et al., 1996; Gillanders and Kingsford, 1996), scales (e.g. Coutant and Chen, 1993, Wells et al., 2000), eye lenses (e.g. Dove and Kingsford, 1998) and soft tissues (e.g. Beauchemin et al., 1988; Ishii et al., 1991; Hellou et al., 1992).

Sample-specific differences in elemental concentrations in tissues other than otoliths have also been reported (e.g. vertebrae—Mulligan et al., 1983; Behrens Yamada et al., 1987; scales—Bagenal et al., 1973; Lapi and Mulligan, 1981; eye lenses—Dove and Kingsford, 1998; soft tissue—Hellou et al., 1992). Such sample-specific differences in trace elements provide evidence for the nonmixing and segregation of populations. Otoliths are an ideal natural marker of elemental composition because they grow throughout the life of the fish and once the elements are deposited, they are unlikely to be resorbed or altered (Campana and Neilson, 1985). Recently, Dove and Kingsford (1998) found that eye lenses may be suitable for differentiating populations of fish because the eye lens has no efficient mechanism for removing ions from the tissue. Eye lenses also grow by the addition of protein-rich cells to their outer surface. Bone, scales, and other soft tissues (e.g. gills, liver, muscles) will reflect a variety of factors including composition during growth, but resorption and remineralization may occur in some species (Gauldie and Nelson, 1990). The temporal stability of the elemental concentrations in other tissues is therefore questionable (Campana and Gagne, 1995). In some species, however, the market value of the fish is likely to be reduced by removing otoliths and eye lenses; therefore, structures such as scales or spines may be easier to obtain. If scales and spines show similar elemental fingerprints (for fish caught at different locations) to otoliths and eye lenses, then their use in stock discrimination studies may be warranted. The use of scales and spines would also allow sampling without the need to kill the fish, with the result that broodstock could be kept alive and individuals from rare or endangered stocks could be sampled. With the exception of two studies (Dove and Kingsford, 1998; Wells et al., 2000), who compared elemental fingerprints of otoliths with eye lenses and scales, respectively, there have been no comparative studies of more than two structures.

The aim of my study was to compare the elemental fingerprints obtained from different structures (otoliths, scales, eye lenses, and dorsal spines) to determine if there were correlations among structures. Because otoliths are not subject to resorption (but see Mugiya and Uchimura, 1989), their use in studies of stock discrimination appears justified; therefore all comparisons were made between otoliths and the other three structures. These four structures chosen for the present study are also very different in terms of their composition. Otoliths are composed primarily of calcium carbonate, whereas scales and spines are composed primarily of calcium phosphate, and eye lenses, largely of water and structural proteins. There may, therefore, be differences in the affinities of ions for the different structures and this difference could have implications for the elemental chemistry of each structure.

The damselfish *Parma microlepis* is a territorial species, both juveniles (Moran and Sale, 1977) and adults (territory sizes 2–17 m^2, Tzioumis, 1995) remain attached to their natal sites. Therefore it is an excellent model species for this kind of research. Otoliths and eye lenses of *P. microlepis* have been shown to contain microconstituents that can be detected with ICP-MS, some elements of which may be site-specific (Dove et al., 1996; Dove and Kingsford, 1998). Juvenile fish are distinguished from adults on the basis of coloration and are generally less than 3 years of age (Tzioumis and Kingsford, 1999). By comparison adults reach a maximum age of 37 years, and fish larger than 120 mm SL may represent a wide range of age classes (Tzioumis and Kingsford, 1999). Adults will therefore have longer and potentially more variable age-integrated elemental fingerprints than those of juvenile fish.

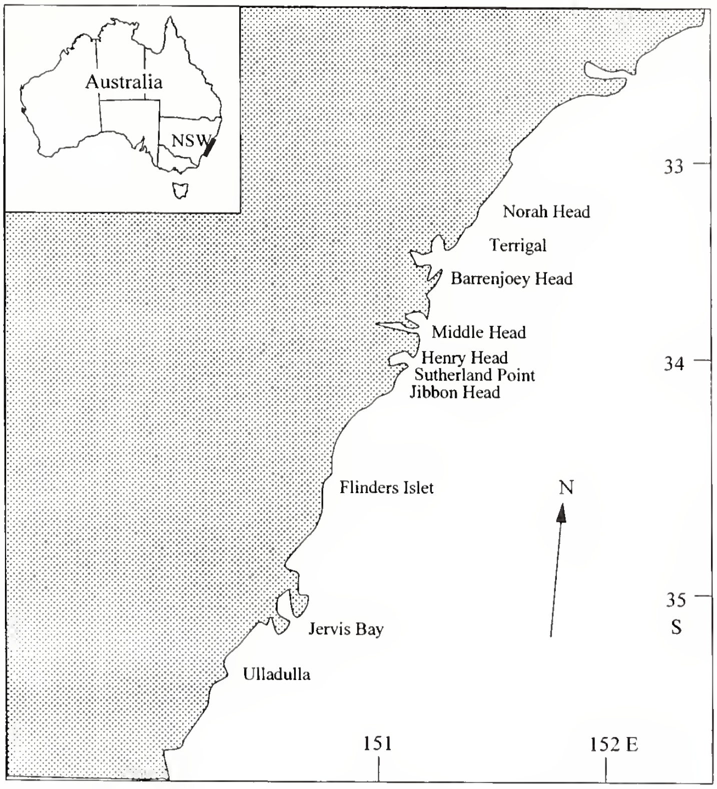

Figure 1

Map showing sampling locations along the coast of New South Wales, Australia.

Materials and methods

Parma microlepis was collected from each of ten locations along the coast of New South Wales, Australia (Fig. 1). Fish were not collected from multiple sites within each location because small-scale differences in trace elements of otoliths and eye lenses (sites separated by 50 to 200 m) were not found in a previous study (Dove and Kingsford, 1998). All fish were collected by divers using SCUBA and a hand-spear between January and April 1998. Between five and seven replicate adult and juvenile fish were collected from each location. Juveniles and adults were distinguished from each other on the basis of differences in patterns of coloration.

Fish were stored on ice during transportation to the laboratory. In the laboratory, the standard length of each fish was measured (Table 1). Otoliths, eye lenses, and approximately five non-regenerated scales were removed from each fish and rinsed in MilliQ water. Otoliths and scales were stored dry at room temperature, and eye lenses were stored at –70°C in Eppendorf microcentrifuge tubes in preparation for ion analysis. Dorsal spines were removed and frozen (–4°C) prior to removal of surrounding flesh. After flesh was removed, spines were also stored dry at room temperature.

Table 1

Summary of collections of *Parma microlepis*. Sample locations are shown in Figure 1. Sample sizes were n=5 for juveniles and adults at all locations, except for adult fish at Henry Head where n=7. Lengths refer to standard length in mm.

Sample no.	Location	Date of collection	Juvenile mean length (range)	Adult mean length (range)
1	Norah Head	Mar 1998	72.0 (56–89)	125.6 (117–133)
2	Terrigal	Mar 1998	83.0 (61–95)	122.6 (105–132)
3	Barrenjoey Head	Feb 1998	64.0 (49–89)	118.8 (106–129)
4	Middle Head	Feb 1998	88.6 (83–94)	125.6 (115–136)
5	Henry Head	Jan 1998	83.6 (71–94)	122.6 (114–136)
6	Sutherland Point	Jan 1998	85.4 (76–93)	118.6 (107–125)
7	Jibbon Head	Mar 1998	83.2 (65–95)	126.4 (114–136)
8	Flinders Islet	Mar 1998	76.0 (73–80)	110.4 (105–124)
9	Jervis Bay	Apr 1998	81.2 (75–95)	117.8 (112–125)
10	Ulladulla	Apr 1998	80.8 (76–90)	111.2 (102–120)

All samples and standards were prepared for ion analysis in a laminar flow cabinet. Otoliths and eye lenses, and scales and spines were individually weighed on an analytical balance (to 0.0001 g) and a microbalance (to 0.001 mg), respectively. All samples were rinsed in 1% nitric acid for 15 s prior to dissolution. Structures were digested in nitric acid (Aristar) either overnight (otoliths and scales) or for 36 h (eye lenses and spines). For eye lenses, sulphuric acid (Aristar) was added following dissolution and each sample was heated to ~90°C for 1 hour. For all structures varying amounts of MilliQ water were then added followed by additional dilution with 1% nitric acid where necessary. This was necessary so that concentrations of the sample solutions were standardized before analysis with ICP-MS.

Samples were analyzed by solution-based ICP-MS (Perkin Elmer SCIEX ELAN 5000). Initially, the four structures of fish collected from one location (Henry Head, Fig. 1) were analyzed in "totalquant II" mode (n=7 fish). This procedure provided a rapid-survey-analysis to semiquantitatively determine the elements present (and their range) within each of the four structures. The instrument was calibrated at six points spanning the mass range from 9 to 209. Accuracy and drift of the machine were determined from spiked samples.

Samples were then analyzed in "quantitative analysis" mode after the instrument was calibrated for either external standardization or addition calibration, depending on the structure and expected concentration range of each element. Measurement parameters for the ICP-MS were as detailed in Gillanders and Kingsford (1996). Seven blank solutions were run at the beginning of each session to determine detection limits, which were calculated from the concentration of analyte, yielding a signal equivalent to three times the standard deviation of the blank signal. Blank solutions were 1% HNO_3 for otoliths, scales, and spines, and 2% acid (1%HNO_3 and 1%H_2SO_4) for eye lenses. Standards were run through the machine at the beginning of each session. Spiked samples were run every ten samples to measure sensitivity changes to the machine through use and for recovery verification.

All samples were initially analyzed by using addition calibration mode. The data were then reprocessed in external calibration mode to obtain concentrations of microelements. Each sample took around 3 to 4 min to analyze, including a delay before starting to read a sample (70 s) and the rinse (1% HNO_3, 0.1% Triton X) between samples of 60 s. Samples from each structure (otoliths, scales, spines, and eye lenses) and size class (juveniles and adults) were analyzed together in blocks, but all samples within each block were randomized to ensure that resulting patterns did not reflect variation in the performance of the instrument.

Univariate and multivariate techniques were used to test hypotheses concerning individual elements and multielement fingerprints of *Parma microlepis*. Pearson's correlations were used to determine the relation between otoliths and each of the other structures for individual elements (Mn, Sr, Ba, and Pb). Separate analyses were performed for each size class and structure.

The match between the otolith microchemistry data and the microchemistry data for each of the other three structures was examined by using two types of nonparametric multivariate analysis. For both types of analysis, the data were standardized by subtracting the mean and by dividing by the standard deviation of the variable, so that each element would have equal weight. A matrix of Euclidean distances among all pairs of samples was then calculated. Mantel's test (from the R package) and the BIOENV procedure (included in the PRIMER computer program; copyright M.R. Carr and K.R. Clarke, Marine Biological Laboratory, Plymouth, UK) were used to determine the degree of correlation between pairs of distance matrices (otoliths and each of the other three structures).

Mantel's test describes the relation between two distance matrices with Pearson's correlation coefficient, r (Mantel, 1967; Legendre and Fortin, 1989). A test of significance for r is obtained by random permutations of the replicate sample units for one of the matrices. A total of

999 permutations were performed for each test and the probability calculated as the number of values equal to, or larger than, the observed value of r divided by the total number of permutations.

If significant relationships were found with Mantel's test, the BIOENV procedure was then used to determine which elements were likely to be important in describing the correlation between the distance matrices (Clarke and Ainsworth, 1993; Clarke and Warwick, 1994). BIOENV calculates rank correlations between a dissimilarity matrix derived from the otolith microchemistry data and matrices derived from various subsets of the elements for either the scale, spine, or eye lens data matrices, thereby defining suites of elements that "best explain" the otolith microchemistry data (Clarke and Ainsworth, 1993). The harmonic or weighted Spearman correlation ρ_w was used.

Results

Otoliths, scales, spines, and eye lenses of *Parma microlepis* differed in composition, both in terms of the actual elements present and the concentration of individual elements. Otoliths, scales, and spines were dominated by calcium, whereas no calcium was detected in eye lenses. The microelement Sr was present in otoliths, scales, and spines at concentrations of 100's to 1000's μg/g of structure, whereas it occurred at low concentrations in eye lenses. Other elements (e.g. Mn, Ba, Pb) were typically found in low-to-trace levels in all structures. Mercury was found in detectable concentrations only in eye lenses.

Univariate analyses

Differences in the concentration of manganese among structures varied over several orders of magnitude (range: 0.05 for eye lenses to 100 μg Mn/g structure for spines). Concentrations of Mn in otoliths showed significant correlations with concentrations of Mn in both eye lenses and scales of juvenile fish, but only with scales from adult fish (Fig. 2, Table 2). For juveniles, fish from one site may have influenced the correlation analyses; therefore fish from this site were removed and the correlations recalculated. This adjustment resulted in a significant correlation between Mn in otoliths and Mn in scales, as found previously. The relation between Mn in otoliths and Mn in spines was also significant when fish from this one site were removed (r=0.302, $P<0.05$). However, there was no longer a significant correlation for eye lenses: fish from one site may therefore have influenced the correlation result for this structure.

Concentrations of Sr in otoliths showed significant correlations with concentrations of Sr in all the other structures for juvenile fish; however the relation was only positive for otoliths and spines (Fig. 3, Table 2). For scales and spines, fish from one site did not unduly influence correlations; when these fish were removed from analyses, correlations were still significant. However, as found for Mn, the correlation between Sr in otoliths and Sr in eye lenses was no longer significant when fish from this site were removed. The only significant relationship for Sr in adult fish was found between otoliths and spines (Fig. 3, Table 2).

Table 2

Correlations between concentration of elements in otoliths and concentration of elements in eye lenses, scales, and spines of juvenile and adult fish collected from ten locations along the coast of New South Wales. Shown are Pearson's correlation coefficients. Significance levels are indicated by * ($P<0.05$) and ** ($P<0.01$), df=48. No corrections were made for experiment-wise error rate; therefore 1 in 20 tests would be expected to be significant by chance alone.

	Otolith			
	Mn	Sr	Ba	Pb
Juveniles				
Eye lenses	0.3410*	−0.3787**	0.1260	
Scales	0.4018**	−0.6237**	0.8810**	
Spines	0.2466	0.6586**	0.9001**	
Adults				
Eye lenses	0.0429	0.2353	0.0239	−0.2564
Scales	0.4717**	−0.0051	0.8839**	−0.1487
Spines	0.2696	0.8446**	0.9121**	−0.0732

Significant positive relationships were found between the amount of Ba in otoliths and the amount of Ba in scales and spines of both juvenile and adult fish (Fig. 4, Table 2). Lead was usually at or below detection limits of the instrument for juvenile fish and therefore correlations were not made. Correlations between the amount of Pb in otoliths of adult fish and the other structures showed no significant relationship (Fig. 5, Table 2).

Multivariate analyses

Mantel's test detected a significant relationship between the Euclidean distances among replicates based on the otolith data and the distances based on data from eye lenses, scales, or spines for juvenile fish (Table 3). For adult fish, however, a significant relation was detected between the otolith data and the scale and spine data (Table 3); thus there may be either changes in assimilation of elements with age or resorption and remineralization may occur in some structures, for example in eye lenses.

The BIOENV analyses showed that the highest rank correlation was found between the otolith data and the scale data for juvenile fish and involved Sr and Ba (Table 3). For adult fish the highest correlation was between the otolith data and the spine data and involved three elements (Mn, Sr, and Ba; Table 3).

Comparisons of juvenile and adult fish for each structure showed no significant relationships (Table 3). The Mantel test, however, was marginally nonsignificant for otoliths (Mantel r statistic 0.2000, P=0.060).

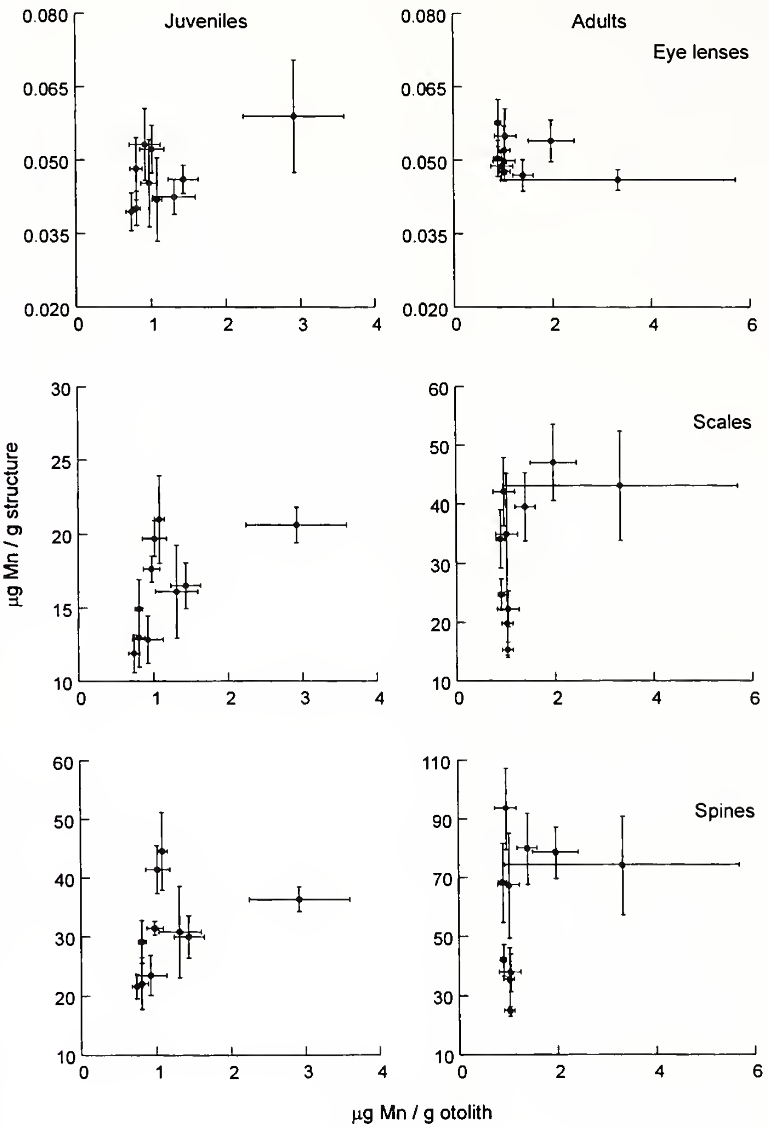

Figure 2

Relation between concentration of manganese in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

Discussion

Otolith elemental fingerprints are reported to be among the most important tools for stock discrimination for some species of fish (Campana et al., 1995). In the current study, adult fish showed a significant relationship between the otolith elemental fingerprints and the scale and spine elemental fingerprints. In contrast, juvenile fish showed significant relationships between comparisons of otoliths and each of scales, spines, and eye lenses. Relationships among some structures were also seen for some individual elements. There have been two other studies involving comparisons between elemental fingerprints of otoliths and other structures (Dove and Kingsford, 1998; Wells et al., 2000). Wells et al. (2000) compared trace elements in otoliths and scales of juvenile weakfish by using correlation analyses on individual elements. They found similar results to those in the current study, namely a significant correlation between Mn, Sr, or Ba in otoliths and that in scales. Dove and Kingsford (1998) compared elemental fingerprints of otoliths and eye lenses. In their study differences between locations were compared by using analysis of similarity (ANOSIM) permutation tests. Where comparisons between locations gave the same response

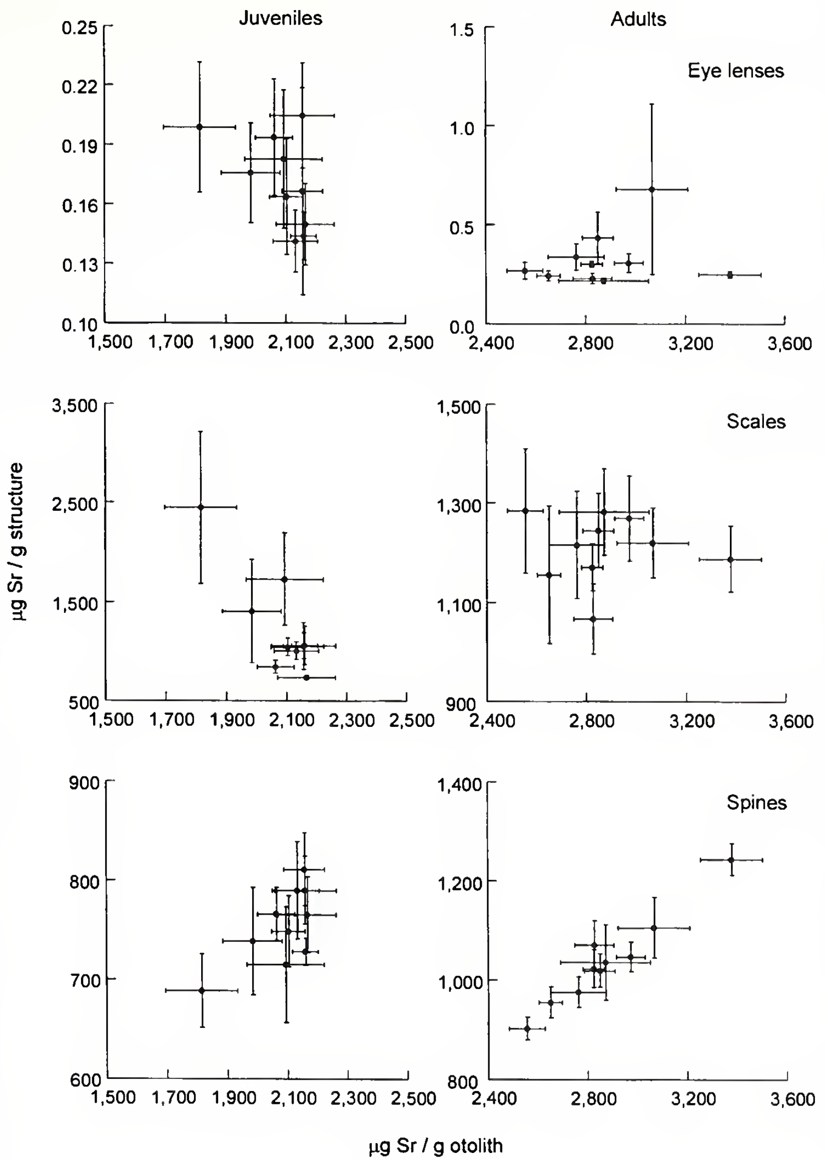

Figure 3

Relation between concentration of strontium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

for both otoliths and eye lenses (either both significant or both nonsignificant), then the two structures were viewed as being similar. With this approach, approximately 73% (11/15) of pair-wise comparisons showed agreement (Dove and Kingsford, 1998). Their approach is, however, indirect and does not show evidence of correlation between data matrices for otoliths and those for eye lenses. I am aware of no other studies that have compared multiple structures for fish obtained from a number of locations.

Otoliths have been considered the structure of choice for determining elemental fingerprints and for relating these to movements or stock structure of fish because otoliths grow throughout the life of the fish and the material of the annual growth increment, once deposited, is unlikely to be resorbed or altered (Campana and Neilson, 1985). Otoliths therefore have the potential to record endogenous and exogenous factors permanently within their calcium-protein matrix. Other structures may also grow throughout the life of the fish (e.g. eye lenses—Dove, 1997; scales—Mitani, 1955; spines—Hill et al., 1989). Although other structures have been used for chemical analyses and for aging fish, some structures are susceptible to resorption and remin-

Table 3

Summary of results from Mantel's test and the BIOENV procedure where otolith microchemistry data were compared with the microchemistry data obtained from eye lenses, scales, and spines. Comparisons are also made between juvenile and adult fish for each structure. The BIOENV procedure (see general text) was done only if significant correlations were found with Mantel's test. Only the combination of elements that contributed to the maximum ρ overall, as measured by the weighted Spearman correlation, are shown for each structure comparison. The significance of the Mantel test statistic is shown by * ($P<0.05$) or ** ($P<0.01$).

Comparison	Mantel r statistic	BIOENV-maximum ρ	Combination of elements contributing to maximum ρ
Juveniles			
Otoliths and eye lenses	0.3402*	0.127	Mn, Ba, Hg
Otoliths and scales	0.5757**	0.587	Sr, Ba
Otoliths and spines	0.4097**	0.403	Sr, Ba
Adults			
Otoliths and eye lenses	–0.0646		
Otoliths and scales	0.3680**	0.454	Mn, Ba
Otoliths and spines	0.4440**	0.512	Mn, Sr, Ba
Adults and juveniles			
Otoliths	0.2000		
Eye lenses	–0.1357		
Scales	–0.0136		
Spines	0.0676		

eralisation (Simkiss, 1974). Despite the possibility of resorption of some elements, otoliths and scales, and otoliths and spines showed a significant correspondence between data matrices for both juvenile and adult fish, which may indicate that scales and spines provide estimates of stock structure that are similar to those obtained from otoliths.

Differences in elemental fingerprints among structures may be due to different routes of ion uptake and differential abilities of each structure to incorporate elements into the organic and inorganic matrix. Calcium and strontium in otoliths are primarily taken up by the gills (Simkiss, 1974), but the route of uptake of trace elements has not been identified (Campana and Gagne, 1995). There may also be some Sr uptake in otoliths through the diet because fish that were fed a Sr-enriched diet showed a detectable increase in Sr/Ca ratios (Gallahar and Kingsford, 1996). Other structures, such as scales and bone, may incorporate ions by diffusion across the gills and through the skin or by ingestion of food and water (Simkiss, 1974). Whether some structures show differential abilities to incorporate ions through different methods of absorption and whether ions are resorbed from different structures in equal proportions is not known.

Otoliths and eye lenses showed the greatest differences in elemental fingerprints between structures. Otoliths are predominantly $CaCO_3$ (Ca constitutes from 30% to 39% of otoliths, Thresher et al., 1994; Dove et al., 1996), although small amounts of protein also occur (Degens et al., 1969) and therefore otoliths are likely to incorporate ions that are able to substitute for Ca in the $CaCO_3$ matrix or bind to proteins in the organic matrix (Gunn et al., 1992; Sie and Thresher, 1992). The spaces between these matrices may also trap ions. In contrast, eye lenses are composed largely of water and structural protein, the latter of which may be soluble or insoluble crystallin (Nicol, 1989). The proteins of eye lenses are rich in sulfhydryl groups that may covalently bind with metals and the proteins also have specific sites for binding with cations (Sharma et al., 1989). Scales and bone are more similar to otoliths in that the dominant ion is Ca (e.g. in bone Ca may constitutes from 24% to 37%; Hamada et al., 1995), but they vary considerably in that otoliths are primarily carbonate structures, whereas scales and bone are primarily phosphate (or hydroxyapatite) structures. Some differences in elemental composition of otoliths and scales or spines may therefore be expected. Because otoliths, scales, and bone are composed predominantly of a mineral, rather than an organic matrix, it is not surprising that these structures were similar in their elemental fingerprints and that eye lenses presented a different fingerprint.

Differences in elemental fingerprints between juvenile and adult fish were also found for all structures. Eye lenses are thought to have no efficient mechanism for removing ions, but there may be changes in structural proteins with age that possibly alter affinities of proteins for specific ions (Dove, 1997). Such ontogenetic effects may result in different elemental fingerprints between juvenile and adult fish for eye lenses. Both scales and spines show some evidence of resorption or remineralization over time in some species. For example, early growth increments in spines of several species are known to be destroyed as the core-matrix expands (Hill et al., 1989; Gillanders et al., 1997) and there is evidence for resorption in scales of fish living in a stressed environment (Sauer and Watabe, 1989).

If an alternative structure to otoliths is required for stock identification, as it may be for broodstock, and for rare or endangered species, or if removing otoliths decreases

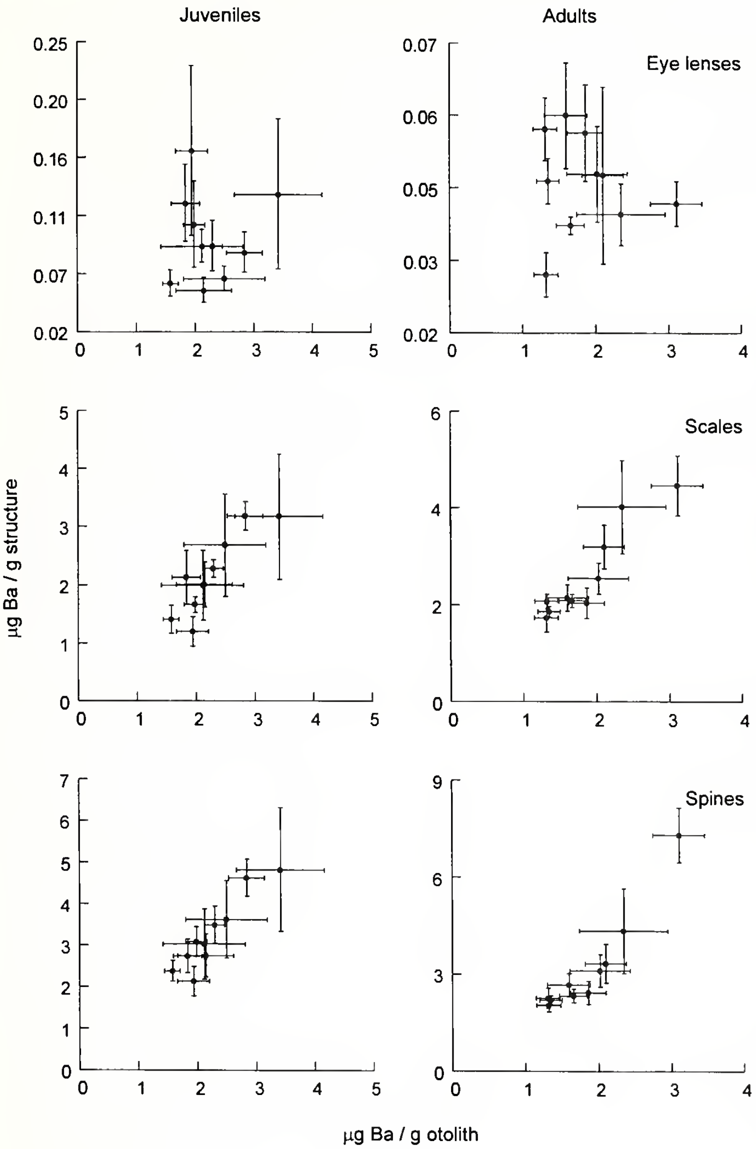

Figure 4

Relation between concentration of barium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

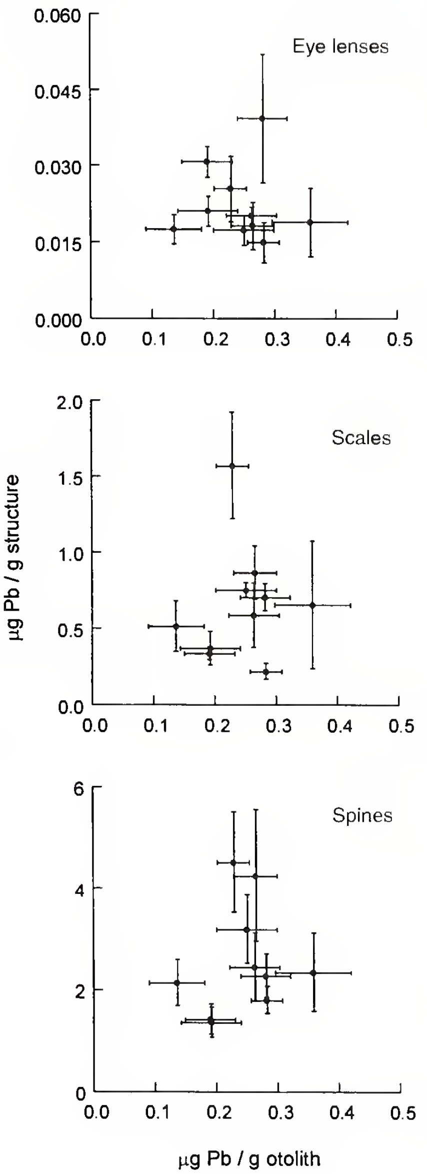

Figure 5

Relationship between concentration of lead in otoliths (mean ±SE) and in eye lenses, scales, and spines of adult fish (mean ±SE) collected from ten locations along the coast of New South Wales. Juveniles are not shown because concentrations of lead were below detection limits.

the market value of the fish, then it is recommended that either scales or spines are the best alternative structure because these were the structures that were significantly correlated with the otolith elemental data for both juvenile and adult fish. However, before using either of these structures, a number of fish (e.g. 30–50) should be collected from three to four locations and the alternative structure, as well as otoliths, should be analyzed. Classification models should then be developed by using data from each structure and the error rates should be compared. This was beyond the scope of the present study because sample sizes from each location were relatively small.

Scales and spines may offer certain advantages over otoliths because they provide a nonlethal alternative. In addition, they can be collected relatively quickly and easily. Scales may also require less preparation for analyses than either spines or otoliths. Thus, scales and spines may provide an alternative structure for determining stock identity.

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Estimating the number of fish in Atlantic bluefin tuna (*Thunnus thynnus thynnus*) schools using models derived from captive school observations

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Abstract—Aerial photographic assessment is a promising technique that could be structured to yield a fishery-independent index of abundance for Atlantic bluefin tuna, *Thunnus thynnus thynnus* (ABT). The accuracy of this approach may be increased by incorporating the relationship between the surface characteristics of a school and the total number of individuals. Our objective was to develop models to facilitate the estimation of number of fish in ABT schools from aerial photographs.

Video cameras were used to observe 74 incidences of schooling for 50 captive ABT approximately one meter in length. Relationships between the surface characteristics of ABT schools and the number of fish in the school were explored by using least-squares regression. The schools ranged in number from 2 to 45 individuals. A weighted regression model incorporating the number of fish in the school at the surface as the independent variable and the number of fish in the remaining portion of the school yielded an r^2 of 0.74. A second weighted multiple-regression model incorporating the number of fish in the school at the surface and in the second depth interval (0–25% school depth below surface layer) of the school as independent variables, and the number of fish in the remaining portion of the school as the dependent variable, with 1/variance as the weight, achieved an r^2 of 0.70. A third model using the length and width of the surface layer of the school as the independent variables and the number of fish in the school as the dependent variable had an r^2 of 0.86. One data point from a wild school is currently available to verify model predictions. This school of 125 individuals is well outside the range of school sizes used to construct the model (2–45 individuals), yet differs from model predictions by only 7%.

We believe that these models have the potential to improve an abundance index based on aerial photographs by estimating the number of individuals in wild ABT schools from surface characteristics observed in aerial photographs.

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Fish. Bull. 99:420–431 (2001).

The bluefin tuna (*Thunnus thynnus*) is distributed worldwide in temperate and subtropical seas. It has a limited distribution in the southern hemisphere. Endothermy by means of vascular heat exchangers allows bluefin tuna to inhabit a wide thermal niche and therefore wide geographic and depth ranges (Carey and Teal, 1969; Carey and Lawson, 1973). In the western Atlantic Ocean, the Atlantic bluefin tuna (ABT), *Thunnus thynnus thynnus,* is distributed from Labrador to Brazil, including the Gulf of Mexico and Caribbean Sea. Adult ABT occur throughout the entire range, but smaller bluefin tuna (less than 45 kg) are not observed frequently above the latitude of Cape Cod, Massachusetts. The Atlantic bluefin tuna is epipelagic and usually oceanic but appears near the coast seasonally (Squire, 1962; Collette and Nauen, 1983) to feed on concentrated assemblages of prey. Adult ABT may attain a length of four meters and a body mass of 680 kg. Large medium (178–195 cm FL) and giant (>195 cm FL) bluefin tuna are targeted by commercial purse-seine, long line, and hook-and-line fisheries (Mather, 1974; Figley, 1984). A recreational hook-and-line fishery (Mather, 1974; Figley, 1984) targets all sizes of bluefin tuna as they appear along the east coast of the United States and Canada from June to October (Mather, 1962).

The combination of changes in the spatial distribution over time and associated uncertainty regarding the independence of eastern and western Atlantic stocks makes the estimation of ABT stock size particularly problematic. The stock assessment for this species has been based upon landings data and abundance indices (Scott et al., 1993). Use of ABT landings data to generate an abundance index may lead to bias due to variability in effort, improvements in fishing technology (Lo et al., 1992), and variability in annual geographic distribution linked to prey distribution.[1] These characteristics of the northwest Atlantic bluefin tuna fishery, in conjunction with population-level behavioral characteristics observed for similar tuna species, suggest that the use of catch-per-unit-of-effort (CPUE) data to evaluate tuna population trends could lead to inaccurate estimates (Clark and Mangel, 1979). The accuracy of CPUE-based assessments in estimating the abundance of bluefin tuna in the northwest Atlantic remains controversial (Clay, 1991; Suzuki and Ishizuka, 1991; Safina, 1993). An extensive discussion of the issues involved in Atlantic bluefin tuna assessment can be found in the National Research Council report by Magnuson et al. (1994).

Recent investigations have focused on the feasibility of using aerial photographic assessment of large medium and giant bluefin tuna in New England waters and in the Straits of Florida (Lutcavage and Kraus, 1995; Lutcavage et al., 1997) as an alternative fishery-independent method of obtaining indices of abundance. The aerial survey method has been used to determine the relative abundance for other pelagic fisheries worldwide, including *Engraulis mordax* (Lo et al., 1992), *Engraulis mordax*, *Sarda chiliensis*, *Trachurus symmetricus*, etc. (Squire, 1972), *Trachurus declivis*, *Katsuwonus pelamis*, *Arripis trutta*, *Thunnus maccoyii* (Williams, 1981), *Mugil* spp. (Scott et al., 1989), and Squire (1993) has reported aerial survey data for *Thunnus thynnus orientalis* and other species. Abundance estimates derived from an aerial assessment are based on biomass or number of individuals per unit of area.

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[1] Chase, B. C. 1995. Preliminary report of the Massachusetts bluefin tuna investigation: the diet of bluefin (*Thunnus thynnus*) off the coast of Massachusetts. Massachusetts Division of Marine Fisheries. Salem, MA 01947, 39 p.

Lutcavage and Kraus (1995) concluded that the aerial method could provide area-specific minimum abundance and distribution data for large medium and giant Atlantic bluefin tuna under good viewing conditions. However, many difficulties associated with aerial photographic assessment of ABT remain to be resolved. Sea state, lighting conditions, and turbidity all play an important role in the ability to detect and produce useful photographs of schools (Lutcavage and Kraus, 1995). Rough seas, sun glare, and high turbidity may all result in reduced detection of schools, limiting the days on which this type of survey method is effective. Visual counts of individuals at the surface derived from aerial photographs are difficult to interpret without a verification count and information on behavioral factors such as surfacing frequency (Lo et al., 1992) and the proportion of the school visible at the surface (Lutcavage and Kraus, 1995). In addition, variability in population movements and distribution could lead to an inaccurate abundance estimate if an intensive, spatially expansive sampling scheme is not employed.

We propose a technique to address the problem of estimating the number of fish in a school (NFS) from the surface characteristics of a school. If the relationship between the surface structure or the surface number of fish and number fish in total school was known, school surface counts from aerial photographs or visual observations could be adjusted to include an estimate of total NFS, facilitating an improvement of area-specific minimum abundance estimates based on visual or photographic data sources.

Atlantic bluefin tuna are believed to exhibit the most rigidly defined spatial structure of schooling fishes (Partridge et al., 1983). Distinct two- and three-dimensional school structures have been described by previous authors (Partridge et al., 1983; Lutcavage and Kraus, 1995). Parabolas and echelons are the shapes of commonly observed surface-oriented two-dimensional schools, whereas the densely packed dome is the shape of a frequently observed three-dimensional school configuration (see Partridge et al., 1983 and Lutcavage and Kraus, 1995 for illustrations). The number of fish observed in two-dimensional surface schools is generally less than 15, whereas three-dimensional schools such as those forming densely packed domes usually have greater than 15 individuals (Partridge et al., 1983). Although the three-dimensional component of bluefin tuna school structure has been observed (Lutcavage and Kraus, 1995), quantitative description and analysis is lacking and little is known of the relationship between the two-dimensional surface structure and three-dimensional structure (e.g. total count, biomass) of schools (Partridge et al., 1983; Lutcavage and Kraus, 1995). In addition, the behavioral and environmental factors that may influence tuna school structure and dynamics remain poorly described (Mather, 1962; Clark and Mangel, 1979; Partridge et al., 1983).

Our study presents a functional relationship between the surface characteristics of and the total number of individuals in ABT schools. We analyzed video-taped footage of 74 incidences of schooling in a group of captive ABT to quantify the relationship between the number of fish visible at the surface and the total number of individuals in the school (NFS), the relationships between school dimensions (e.g. length, width) and NFS, and to explore the effect of environmental conditions within the net-pen enclosure on school size and dimensions. We also analyzed the vertical distribution of individuals within schools across school size, and propose a mechanistic explanation for the limited size of the two-dimensional schools observed by Partridge et al. (1983) and Lutcavage and Kraus (1995). We then apply the predictions from one of the resultant models to the single open-ocean school size estimate available.

Methods

Field methods

We employed a 30.5-m diameter, 15.3-m deep, cylindrical floating net-pen enclosure (Fig. 1) to hold the tuna used in our study. This enclosure is similar to those used in tuna research and culture operations around the world. Its low cost, large internal volume (11,128.5 m^3), and its resiliency to dynamic and often damaging effects of the offshore environment make this enclosure the most appropriate type for observing the behavior of large pelagic fish in captivity. The enclosure proved to be very resilient to the damaging effects of a close pass of a hurricane and a tropical storm. A white, one-inch, straight-hung mesh net constituted the vertical walls and bottom of the enclosure.

The enclosure was anchored 32.2 km offshore of Wachapregue, VA, on the southwest corner of 20 Mile Hill—a bathymetric feature that rises within 33.5 m of the ocean surface in deeper surrounding waters. This location is relatively near shore and close to a temporally and spatially reliable aggregation of small (~1 m) Atlantic bluefin tuna regularly targeted by recreational fishermen.

The vertical temperature profile (°C), dissolved oxygen (mg/L), pH, suspended solids (NTU), Secchi depth (m), and conductivity (ppt) were monitored twice daily inside and outside the enclosure at 3-m intervals to a minimum depth of 15 m.

A pattern of seven, single-hook trolling lures were fished from a 18.3-m commercial vessel on 13-kg or 22-kg class trolling gear to capture fifty bluefin tuna in the vicinity of the study enclosure in June and July 1996. Tuna were subdued as quickly as possible and landed in a specialized cloth stretcher. We recorded the fork length (cm), approximate weight (kg), and general condition of each fish and released the fish into a 2400-liter elliptical transport tank. Compressed, bottled oxygen was employed to elevate

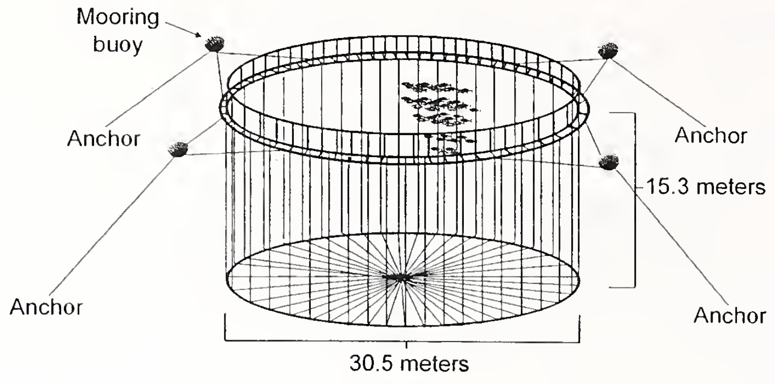

Figure 1

Illustration of the study enclosure. The tuna are scaled in relation to the enclosure. Spatial relationships are slightly distorted owing to the three-dimensional perspective of the illustration.

the levels of dissolved oxygen within the transport tank to ease the physiological stress associated with strenuous activity. Fresh seawater was continuously pumped into the tank to eliminate metabolic waste and maintain water quality. The tank had a padded top that minimized the potential for injury to fish during transport and reduced water loss resulting from boat movement. Viewing ports in the padded top allowed observation of the specimens while in transport. Physical contact with individual tuna was minimized, and when contact was necessary, only well-padded devices were employed. Transport time of individual fish was variable, but generally less than 3 hours. The number of fish simultaneously transported in the tank was controlled to avoid crowding and the depletion of dissolved oxygen. The tuna were recovered from the transport tank by using a cloth stretcher and released into the net-pen enclosure. Divers in the enclosure released the tuna individually, ensuring that they recovered proper spatial orientation upon release. Released specimens were assimilated quickly into the existing shoals of captive fish.

Video cameras (Hi8) in waterproof housings were used to record school structure of the captive tuna. A shutter speed of 1/2000 second was used to optimize the resolution of still frames within the constraint of available subsurface (<15.3 m depth) light. The automatic focus feature of the camera was disabled to avoid rapid fluctuations in focal depth from the intended subject to particulate matter suspended in the water column. Observations of schooling tuna were recorded with stand-alone cameras mounted inside the enclosure and with hand-held cameras during observation dives. Cameras mounted to the floating net pen on specialized polyvinyl chloride pipe structures were stabilized with elastic compensators to lessen movement caused by wave energy. Mounted cameras were positioned to provide head-on and subsequent perpendicular views in relation to the axis of motion of a school. This filming strategy allowed a more accurate observation of the characteristics of the school in three dimensions. Schooling was recorded more efficiently by using hand-held cameras during dives than through use of mounted cameras. During a dive, the entire internal volume of the enclosure was often visible from a given point, allowing a diver to anticipate the path of travel of a tuna school and to reposition the camera to attain the best possible images. Regardless of the apparent ease of tuna schools, divers positioned themselves against the enclosure's external wall to ensure minimal behavioral modification in the filmed schools. Video recordings from both mounted cameras and from held cameras were used in analysis.

Laboratory video analysis

Video recordings of ABT schools were reviewed, and 74 incidences of schooling in which an entire target school was visible were used for further analyses. All observations took place during daylight hours (0900 and 1600 h) and none took place during or within one hour of feeding events. An image analysis system that allowed digitization of points directly from a (paused) video source was employed for more precise quantification of school characteristics (Fig. 2). The system employed a video scan converter that overlaid the image output from a computer video source (640×480 pixel resolution) upon the video source image. The video scan converter allowed the user to select a color in the computer video overlay to be made transparent, revealing the underlying video signal (Fig.2). When employed in conjunction with an image analysis software package (SigmaScan Pro) on a personal computer, all the features of the image analysis software could be used on any still (paused) video source image without the use of a video frame-grabber. The position of each individual fish and school depth intervals were delineated by using the graphic capabilities and the Cartesian coordinate system of the image analysis software. The positions of individual fish in the school could be marked while the recording was advanced or reversed frame by frame, allowing the identification of poorly illuminated fish or fish that may have been hidden by individuals in the foreground of the school.

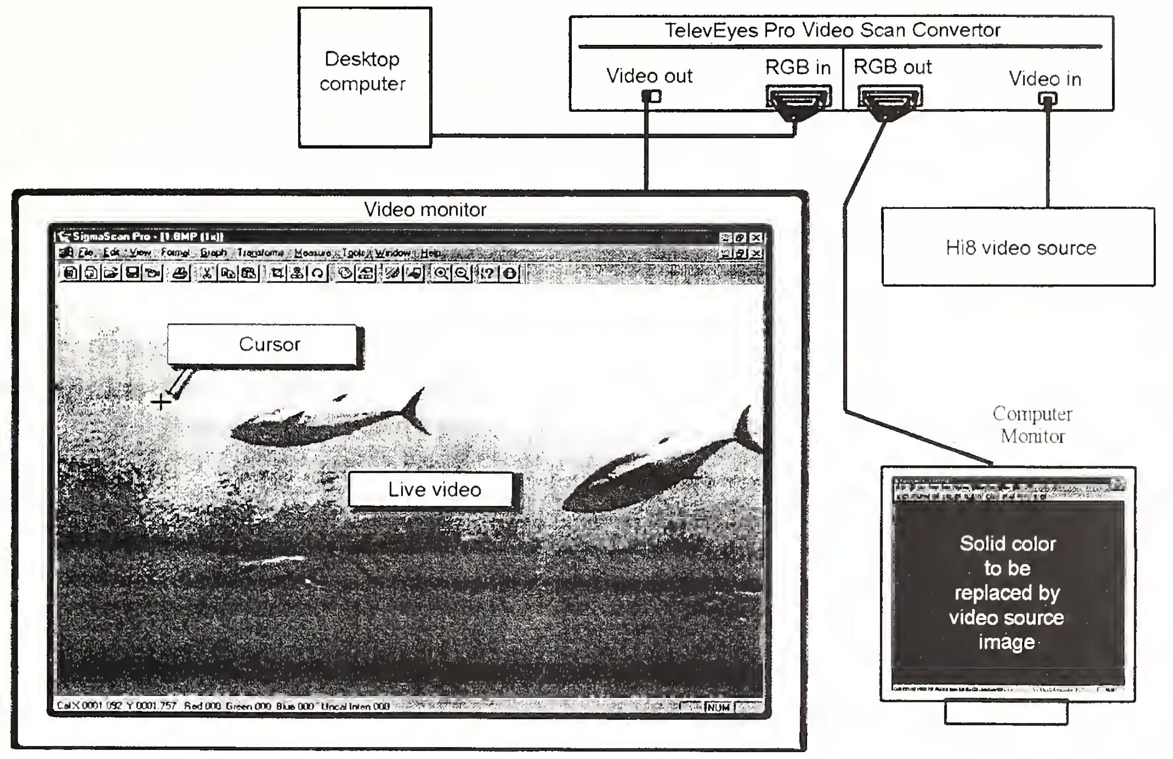

Figure 2

A schematic representation of the configuration of the video equipment used for our analyses.

Data analysis

School size Groups of tuna were considered to be schools and were included in the analyses if they were a polarized group (multiple individuals maintaining lateral proximity to neighbors and actively maintaining the same direction of travel during an observation period). The total number of fish in each school was counted and the frequency distribution of school size was determined. Because the observations were assumed to be independent and the total sample size was less than 2000, the normality of the distribution was tested by using the Shapiro-Wilks W-test. Least-squares regression was then used to evaluate the relationship between school size (N_s) and each environmental variable.

Predicting number of fish in school from surface counts Our video footage was filmed at an oblique perspective to the upper boundary of schools occurring within two meters of the water's surface. Measurement of school characteristics in body lengths or meters was not possible because of the camera angle or because of poor image resolution due to low light or high turbidity level. The total number of individual fish (N_s) was determined and the distribution of individuals within the school was described in terms of five depth intervals (Fig. 3). The surface interval included fish that were at the immediate surface of the school or fish that overlapped other fish at the surface on the horizontal plane. Each of the four subsequent intervals encompassed 25% of the remaining depth of the school. The number of fish per interval was designated as N_i ($_i$=the number of tuna in the i^{th} depth interval). Fish positioned at an interval boundary were assigned to the interval in which the greater portion of their body volume was positioned. Analysis of covariance (ANCOVA) (Sokal and Rohlf, 1995) was employed to detect differences in the slopes of each of the regressions of N_i on N_s in order to determine whether the distribution of individuals into school depth intervals changed in proportion to school size.

Three individual least-squares regression models were used to predict school size. The relationship between the number of individuals in the surface interval (N_1; independent variable) and the number of individuals in the remainder of the school (N_S; dependent variable) was first explored using simple least-squares regression (Sokal and Rohlf, 1995). The distribution of N_S given N_i was heteroscedastic necessitating the use of a weighted least-squares regression model by using a weight of 1/variance (Kleinbaum et al., 1988). A similar-weighted multiple-linear-regression relationship between N_1, N_2 (independent variables), and N_S (dependent variable) was developed because fish below the immediate surface of the school are sometimes seen and counted in photographs.

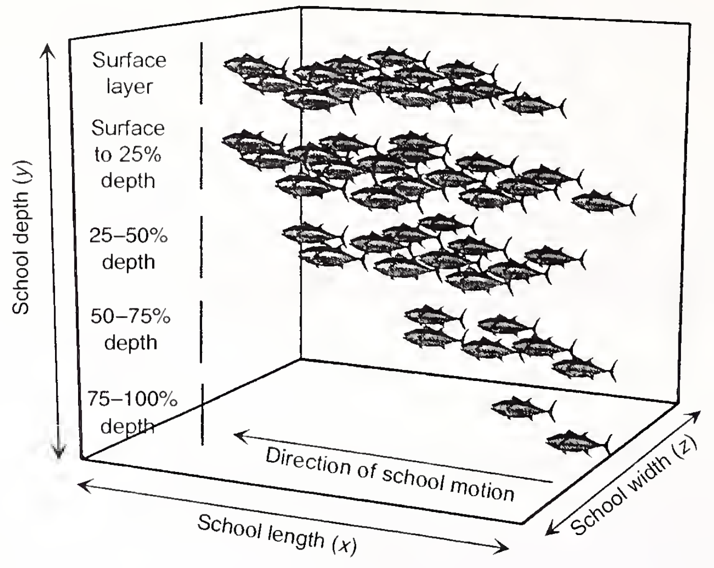

Figure 3

Representation of a school of 50 Atlantic bluefin tuna. Individuals have been separated into depth intervals according to the mean values for all school sizes. Layers are arbitrarily separated in the figure to allow visualization of school structure.

Predicting number of fish in school (NFS) from school dimensions School length, width, and depth in number of individual fish were recorded as indicators of school shape (Fig. 3). As in the prediction of NFS from surface counts, measurements were recorded in number of fish, not metric distance, because a precise spatial scale could not be established consistently. The lack of an accurate spatial scale also precluded the measurement of fine-scale school structure such as interindividual distances. School dimension measures were recorded according to the movement axis of a school. Length in number of fish was measured along the axis of school motion (x); depth was measured vertically (y) and width (z) was measured perpendicular to x on the horizontal plane (Fig. 3). School length and width were analyzed in relation to school size by least-squares regression. Regression models employed each one or both dimensions (i.e. length, width, length and width) of school shape as independent variables, and N_S as the dependent variable. Depth data were not analyzed in relation to N_S because these data may not be collected practically from wild schools. The relationship between dimensions of each school and selected environmental variables was examined by using least-squares regression.

Results

Behavior of the specimens

The captive specimens used in our investigation exhibited a high degree of awareness of the walls of the enclosure, even during periods of excited behavior. No collision with or brushing of the net wall was observed from above the surface or in the analysis of diurnal activities from underwater video footage, and no evidence of nocturnal collisions was observed. After a brief period of acclimation, the tuna did not actively avoid divers in the enclosure; they reacted only to avoid collision.

Visualizing the model

A three-dimensional model of the typical structure of a school of ABT was constructed based on the mean characteristics of the schools analyzed and on qualitative observations of school structure (Fig. 3). The proportionate distribution of individuals within school depth intervals varied little (see ANCOVA results below), suggesting that a single model adequately describes the mean vertical distribution of individuals for schools of varying size.

Number of schools and NFS

When a single school comprised the entire group of 50 tuna, less than approximately 20% of the enclosure volume was involved in containing such a school (senior author, pers. obs.). Fish swimming within such a school were observed to travel along a slowly arcing path around the entirety of the enclosure without making sharp turns. Smaller schools (up to 25 individuals) occupied only a very small portion of the volume of the enclosure. Single large schools separated into two or more smaller schools and joined back together with fluidity. When more than one school was observed simultaneously, each school exhibited movement independent of another. If two schools came close to one another in the enclosure, they would either pass by, move through the other group, or join together to

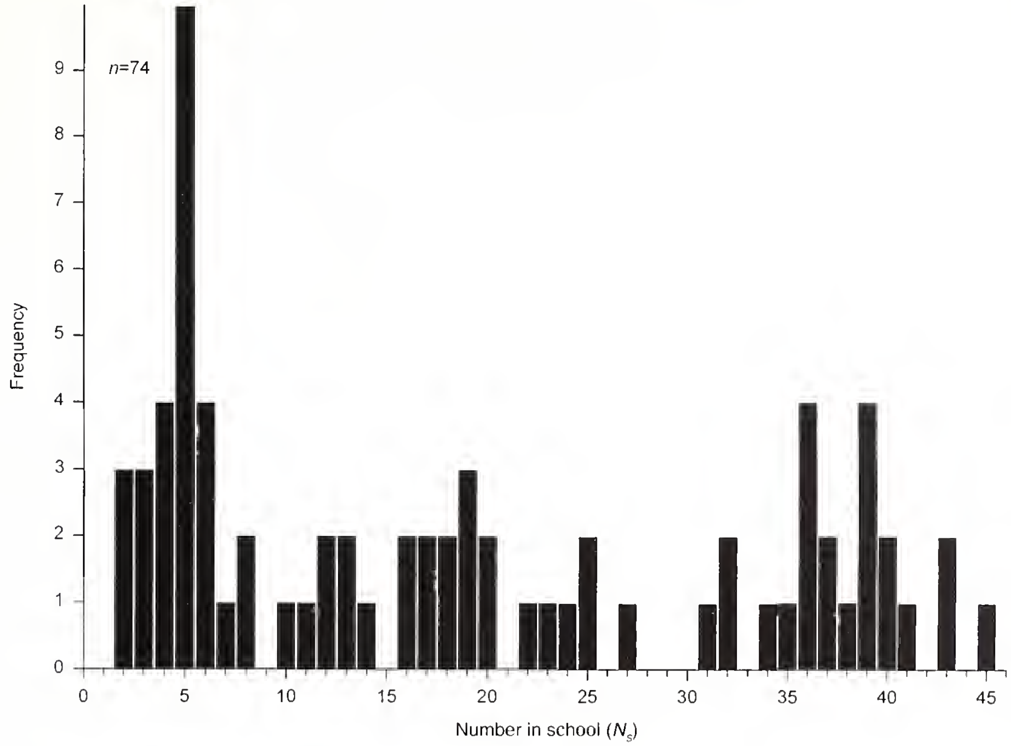

Figure 4
Frequency distribution of school sizes of Atlantic bluefine tuna.

form a larger group. While schools were remixing during such encounters, portions of one group would sometimes join another, maintaining the same number of independently acting groups, but changing the number of individuals within each group.

Particularly in larger schools, individual fish positions were observed to be dynamic, yet the overall shape of the school remained relatively constant. The mean size of schools observed was 18.88 individuals (n=74, SD=13.90). The smallest schools observed had 2 (n=3) individuals, and the largest had 45 (n=1). The frequency distribution of school sizes was not normal (Shapiro-Wilks' W, P<0.0001) and had two prominent modes centered at 5–10 individuals and 35–40 individuals (Fig. 4).

No statistically significant relationships between environmental variables and N_s were observed (Table 1). However, low power due to small sample sizes may have reduced our ability to detect significant effects.

Predicting NFS from surface counts

The relationship between the number of individuals in each depth interval and school size was linear in all cases. Although the r^2 values for each N_i–N_s regression were relatively low, their slopes appeared to be similar and were within a narrow range (0.14–0.23). However, ANCOVA revealed that the slopes were significantly different (P<0.001). Although the slopes were significantly different, the number of individuals in each interval remained in the same proportion except at low school sizes (<15 individuals).

The regression model incorporating the number of fish in the surface interval of the school as the independent variable and the number of individuals in the remaining portion of the school as the dependent variable had an r^2 of 0.67 (P<0.0001) (Eq. 1, Table 2). However, this regression model is likely biased owing to heteroscedasticity in the dependent variable. A second least-squares regression model, incorporating a weight of 1/variance, achieved an r^2 of 0.74 (P<0.0001) (Eq. 2, Table 2). The third regression model incorporated the number of fish in the surface interval (N_1) and the second interval (N_2) of the school as independent variables, the number of fish in the remaining portion of the school as the dependent variable, and 1/variance as the weight. This model had an r^2 of 0.70 (P<0.0001) (Eq. 3, Table 2). Partial F-tests for these models could not be executed because the dependent variable N_s–(N_i+ . . . N_i) changed depending on the number of school depth intervals used to predict NFS.

Three least-squares regression models were used to predict school size from school length and width. The model using length as the independent variable and N_s as the dependent variable had an r^2 of 0.74 (P<0.0001) (Eq. 4, Table 2). The second model predicted N_s from school width, achiev-

Table 1

Mean, minimum, maximum, and standard deviation of environmental data (pH; °C=degrees Celsius; mg/L=milligrams/liter; ntu=turbidity units; ppt=parts per thousand) recorded during schooling observation periods. The r^2 and P-values for linear regressions of each school measure on each environmental variable are provided. Measurements of dissolved oxygen were discontinued due to instrument failure.

Date	Observation start time	pH	Temp. (°C)	Dissolved oxygen (mg/L)	Total suspended solids (ntu)	Conductivity (ppt)
27 Jun 1996	10:00 AM	—	—	—	—	—
21 Jul 1996	3:30 PM	8.51	22.90	9.37	—	27.45
6 Aug 1996	10:11 AM	8.42	22.18	8.62	0.12	28.65
7 Aug 1996	8:45 AM	8.43	24.18	8.39	0.23	28.98
15 Aug 1996	3:45 PM	8.44	23.84	—	0.60	28.06
17 Aug 1996	10:48 AM	8.46	23.86	—	0.77	28.42
22 Aug 1996	11:55 AM	8.47	23.40	—	0.05	27.63
23 Aug 1996	11:15 AM	8.50	23.50	—	0.45	29.63
Mean		8.46	23.41	8.79	0.37	28.40
Minimum		8.42	22.18	8.39	0.05	27.45
Maximum		8.51	24.18	9.37	0.77	29.63
Standard deviation		0.04	0.68	0.52	0.28	0.76
	N_s	0.1*	0.15*	0.84*	0.09*	0.04*
Coefficient of determination	School length	0.01*	0.19*	0.91*	0.02*	0.01*
	School width	0.09*	0.22*	0.46*	0.1*	0.03*

* $P>0.10$.

Table 2

Regression equations employed to predict school size. The results of partial F-tests indicated that model 6 is the best predictor of school size (N_s). N_1, N_2, school length, and school width were measured in individual fish.

Model	Equation	n	r^2	partial F
1. N_1 vs. N_s	$N_s = (0.0337867 + 3.3173368 \times N_1) + N_1$	74	0.67*	
2. N_1 vs. N_s (weight=1/variance)	$N_s = (0.2042865 + 3.1849359 \times N_1) + N_1$	74	0.74*	
3. N_1, N_2 vs. N_s (weight=1/variance)	$N_s = (-1.412557 + 1.8516536 \times N_1 + 0.4190831 \times N2) + N_1 + N_2$	74	0.70*	
4. *Length* vs. N_s	$N_s = -6.769737 + 5.5334126 \times length$	74	0.74*	
5. *Width* vs. N_s	$N_s = -6.70494 + 5.7894971 \times width$	74	0.79*	
6. *Length, width* vs. N_s	$N_s = -9.788046 + 3.6463236 \times width + 2.7083604 \times length$	74	0.86*	(6 vs. 4) * (6 vs. 5) *

* $P<0.0001$.

ing an r^2 of 0.79 ($P<0.0001$) (Eq. 5, Table 2). The final, multiple regression model used school length and width to predict N_s (Eq. 6, Table 2, Fig. 5) with an r^2 of 0.86 ($P<0.0001$). Partial F-tests revealed that the final multiple regression model explained significantly more of the variation in school size than either length or width separately. No significant relationships were seen when school dimensions were considered in terms of environmental factors (Table 1).

Discussion

School structure

The non-normal frequency distribution of NFS and the appearance of modes in our data (Fig. 4) suggest the formation of elective groups of particular number. Previous investigations in school size show that fishes actively assemble into elective group sizes dependent upon the

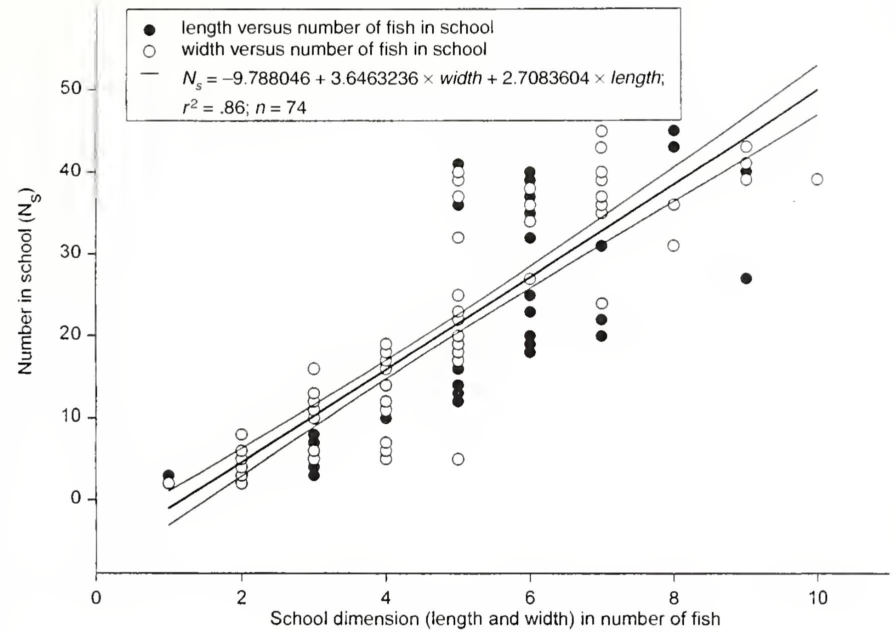

Figure 5

School size predictions based on multiple linear regression of total school number (N_s) on school length (L) and width (W).

interaction of predation risk, food availability, migratory status, and the species' life history (Hager and Helfman, 1991; Pitcher and Parrish, 1993). Elective group size for a species differs continuously under natural conditions with varying abiotic and biotic factors. At a discrete point in time when two schools were present in the enclosure, the size of one group determined the maximum number of individuals that could be in the other. However, there were no barriers to formation of groups numbering anywhere from 2 to 50 individuals. Therefore, the modes in group number that we observed should have resulted from underlying behavioral tendencies rather than enclosure-induced limitation of group size. If elective group sizes form in response to environmental conditions, then the range of environmental conditions during the study period was probably insufficient to detect environmental influences on NFS, and therefore elective group sizes.

The range of school sizes that we observed in the enclosure was limited by the number of individuals available to join schools. Photographs of ABT in the northwest Atlantic Ocean over a 50-day period in 1993 revealed that surface school counts ranged from 5 to 1294 individuals, and that the median school size was 84 individuals (Lutcavage and Kraus, 1995). The median value is well in excess of the maximum number of fish observed in the surface layer of our schools, emphasizing the importance of verifying the accuracy of our model predictions for larger schools with field data. When very large schools occur in relatively shallow water, the vertical depth of the school would be confined by the maximum water depth. A similar effect could be imposed by physical and chemical barriers such as vertical stratification in temperature and dissolved oxygen. Tagging studies may reveal more of the individual and group behaviors of this species in relation to the environment and assist in further understanding the way in which school structure may be affected by the physical and chemical environment.

Our results show that the vertical distribution of fish (in intervals) varies little across the range of observed school sizes. The ANCOVA of N_i on N_s illustrated that the slope of each regression was significantly different from all others (all $P<0.001$), but the biological importance of this difference is questionable because the slopes varied by less than 10%. Although the rate at which individuals are added to each interval varies as school size increases, there is no consistent trend in slopes among intervals. Furthermore, the statistical significance of the difference in slopes may be driven by the small standard error for each regression and extremely high power (>0.99).

The shape of large schools of bluefin tuna was less variable than that of small schools. Schools of less than 15 individuals are less vertically expansive, and generally one to three fish deep; larger (>15 individuals) schools are more than three individuals deep (Fig. 6). The pattern of increasing vertical depth continues to the largest school sizes observed, which are nearly always more than five individuals deep. The weakly cone-shaped profile of the model school depicted in Figure 3 is representative of the shape of most schools with more than 3 intervals. Smaller schools tend to be distributed in a vertically shallow, loosely oval profile. Our findings related to school structure are consistent with the observations of other investigators who have

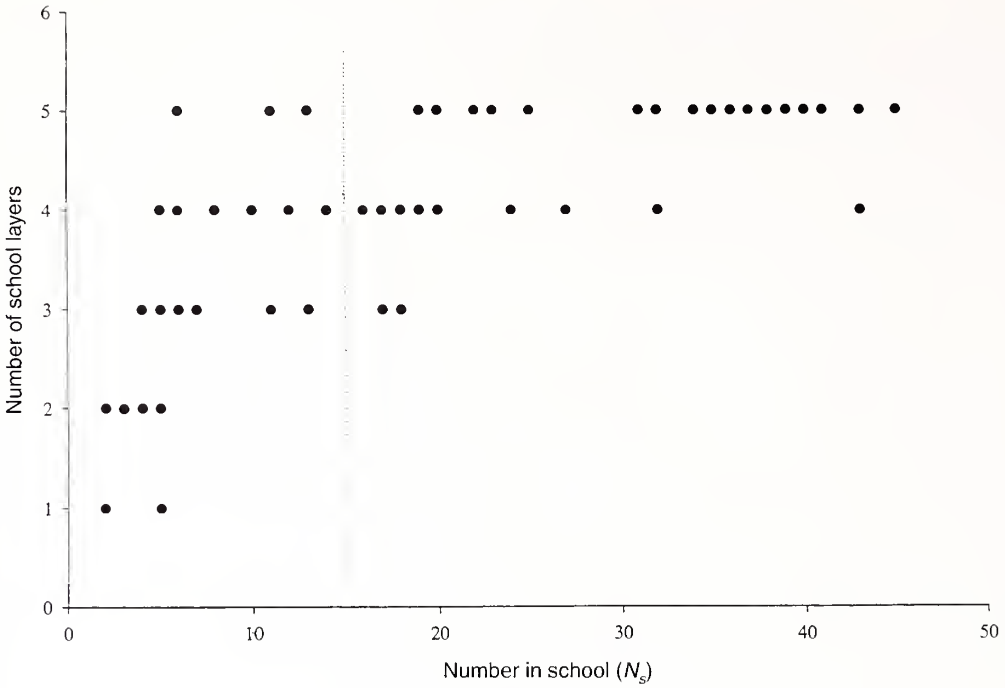

Figure 6

School depth in number of individuals plotted against total school number (N_s). The dotted line is drawn to illustrate the increase in the vertical expanse of schools coincident with the maximum number of individuals observed in parabola and echelon-shaped schools. The maximum number of depth intervals in our analysis was 5. Schools greater than 5 individuals in total depth are described by a surface layer and four 25% depth intervals.

observed that small schools have a strong horizontal aspect, and little vertical expanse. For example, Partridge et al. (1983) and Lutcavage and Kraus (1995) observed that small (<15 individuals) parabola- and echelon-shaped schools of noncaptive giant ABT vary little in shape. Interestingly, parabola and echelon shapes are not observed for large (>15) ABT schools, coinciding with changes in interindividual orientation between groups of less than 10 and 10–20 individuals (Partridge et al., 1983). This difference is similar to the shift in the depth of schools that we observed with approximately 15 individuals (Fig. 6). It is possible that Partridge et al. (1983) and Lutcavage and Kraus (1995) observed small schools in these configurations because larger schools expand vertically and adopt the semiconical shape that we describe. These results suggest that there may be a critical minimum number of individuals that must be present in a horizontal layer before schools begin to expand vertically, which would explain the limited size of two-dimensional ABT schools.

It is possible that ontogenetic variation in school structure exists, but understanding how such changes occur is critical in determining how variability in school structure could affect our modeling approach. Because our models use numerical relationships rather than distance metrics to predict school size, neither ontogenetic shifts in interindividual spacing or packing density changes related to school size should substantially affect the predictive ability of our models. However, if ABT schooling behaviors change at a more basic level due to enclosure or changing fish size, then our estimation techniques may be invalid outside captivity or with larger fish. Basic changes, such as vertical distribution of individuals within schools, the three-dimensional shape of schools, and strong school structure responses in relation to environmental factors, could all have serious effects on our modeling approach. Further, we feel that schools similar in form to those described as "densely-packed domes" by Lutcavage and Kraus (1995) could be described well by our models, but that numerical estimation of other school types might require the application of a different estimation technique.

Environmental effects on school formation

The physical environment may play a role in determining the vertical position of tuna in the water column (Holland et al., 1990; Block et al., 1997) or school structure (Partridge et al., 1983; Lutcavage and Kraus, 1995). No relationship between environmental variables and the shape of schools (quantitatively determined) or the vertical position of fish (in qualitative observations) were observed in our study, perhaps because of the small range and lack of vertical structure in salinity, pH, and dissolved oxygen

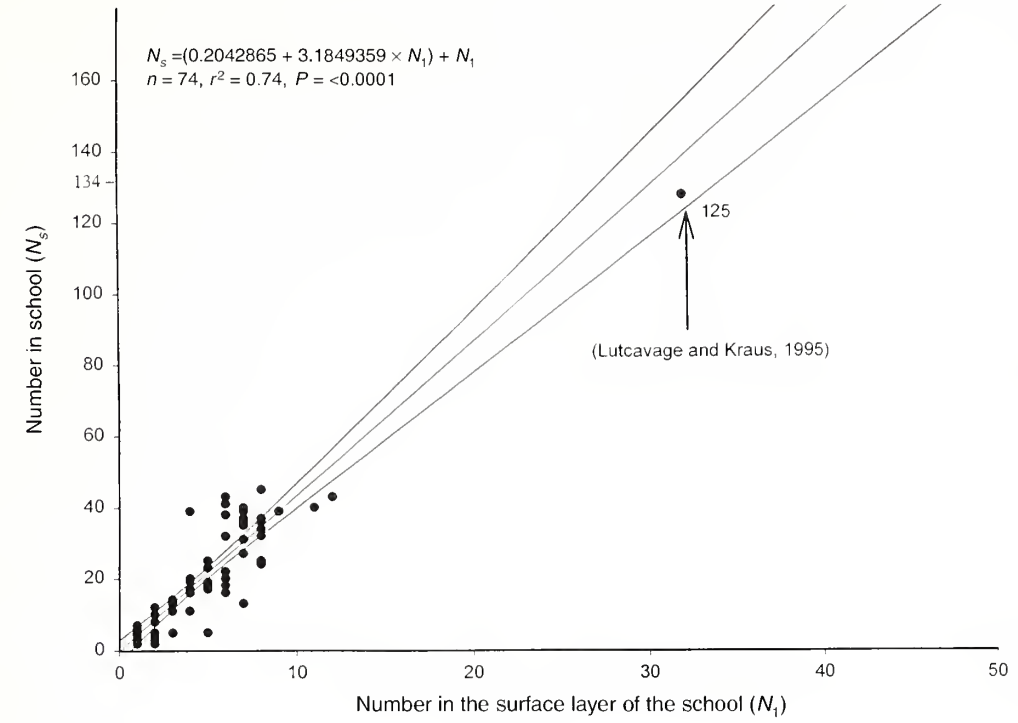

Figure 7

Comparison of school size observations to values predicted by the regression model using number of fish in the surface layer (N_1) weighted by 1/variance to predict school size (N_s). 95% confidence intervals are displayed for reference. The single data point from Lutcavage and Kraus (1995) is included for reference, but was not included in the regression model. The dotted line indicates the predicted value of school size from our model for the numbers at the surface observed by Lutcavage and Kraus (1995).

measurements (Table 1), the time when school structure data were collected, or low statistical power from small sample sizes. However, on a very limited number of occasions, the thermocline became situated within the enclosure at approximately 10 m. Only a few individual fish traversed the thermal boundary, and such excursions were very brief. Entire schools were not observed to cross to the cold side of the thermal boundary. The thermal profile is an important factor in determining the vertical distribution of Pacific yellowfin (*Thunnus albacares*) and bigeye (*T. obesus*) tuna (Holland et al., 1990). It is reasonable to assume that the thermal profile is important in the vertical distribution of Atlantic bluefin tuna as well, and that this effect was not detected in our study because of the periodicity of observations, the limited variation of environmental conditions within the enclosure, or the spatial constraints imposed by the enclosure.

Predicting NFS from surface counts

Our least-squares regression model predicts the number of fish in ABT schools from the number of individuals at the surface of the school without attempting to describe the fine-scale structure within schools in terms of interindividual spacing and orientation. The number of fish in the surface interval alone accounts for 74% of the variation in school size of three-dimensional schools similar to a densely packed dome. Because the only school type that we observed was similar to the densely packed dome, the applicability of our model to three-dimensional schools of other configurations is questionable and may be determined only by studying ocean schools of other configurations. Furthermore, the application of our model to two-dimensional schools such as parabolas, echelons, and surface sheets is not appropriate or necessary given the apparent lack of a three-dimensional component in their structure.

The only data point available to verify our model predictions is from a school described as "dome-shaped" that was photographed and subsequently captured by purse-seine (Lutcavage and Kraus, 1995). Thirty-two fish were counted at the surface of this school from an aerial photograph, and 125 "large giant" ABT were subsequently captured by the purse-seine vessel. We applied our model that predicts NFS from the number at the surface of the school to this datum to produce a NFS prediction. The prediction with our model of 134 individuals differed by 7% from the purse-seine capture of 125 individual fish, and was well within the 95% confidence intervals (Fig. 7) of model predictions. When ABT schools are captured by purse seine, it is assumed that nearly the entire school is captured.[2] If the net intersects an edge of the school while it is being deployed, the entire school will change its direction of travel in unison resulting in either the entire school being encircled in the net and captured or in the entire school escaping into open water. As a result, the estimate of total NFS from this data point is likely to be an accurate count of the number of individuals in the school. It is encouraging that our model so closely estimated the NFS of large giants considering that it was constructed from data for age 2+ fish that are a fraction of the size of large giant bluefin tuna. The accuracy of our prediction indicates the potential for generality of ABT school structure across both tuna size and NFS. However, substantial verification of our models is necessary before they may be applied to abundance estimation.

Predicting NFS from school dimensions

An alternative to using N_1 and N_2 to predict N_s is to use maximum school length and width. Identifying the longest and widest axis of the surface of a school and counting the number of individuals along these axes may yield more accurate estimates of the total NFS. The maximum dimensions of bluefin schools (length, width) had greater power as predictors of N_S than N_1 and N_2 (model 3 versus models 4, 5, 6, Table 2). Moreover, a combination of length and width to predict N_s produced a more confident estimate of school size than models using either variable individually. Irregularity in length and width at small school sizes likely introduced variation that reduced the individual predictive power of these variables. Inclusion of both length and width in the model to predict N_s could allow accurate prediction of small schools that are elongate or wide.

The regression diagnostics for the model using school length and width to predict school size (model 6, Table 2) suggest that it is the more reliable model for estimating NFS with aerial photographs. For the single open-ocean estimate available in the literature (Lutcavage and Kraus, 1995), the length and width of the school in number of individuals could not be determined. Model 6 may have the potential to yield a more accurate estimate of school size with a wider range of photograph qualities (as affected by sea state, water clarity, sunlight, etc.) because of its ability to predict NFS from partial surface counts. However, the utility of school length and width to predict N_s will remain uncertain until field data are available for thorough evaluation.

Enclosure effects

The effects of capture and captivity on school structure and behavior were points of concern in our study. Evaluating the effects that the enclosure had on school structure is problematic because the same factors that led to the use of an enclosure to make the observations preclude a direct *in situ* comparison. Because tuna are highly mobile and unpredictable in their movements, it would be difficult to obtain a number of school observations comparable to that collected from the captive fish in our study. Furthermore, the ability to approach schools in nature without significantly disturbing them is questionable, and effective observation at a distance that would not cause disturbance would be unlikely because of turbidity and the normal movement of schools. In this respect, a group of tuna that has become comfortable with the presence of human observers may be a better source of accurate school structure observations than a school of noncaptive fish that may perceive a human or mechanical presence as a predatory threat and react accordingly. Evidence of the acclimation of the study specimens to the enclosure was seen in their active and aggressive feeding behavior (Hanrahan and Juanes[3]) that was similar to the available anecdotal accounts of their open-water feeding behavior. The relation between fish length and enclosure dimensions may cause the impression that school formation was constrained heavily by captivity and that multiple schools could not achieve meaningful separation from one another (Fig. 1 illustrates a school of tuna at a scale of 1:1 to the enclosure). However, the relatively low density of fish in the enclosure (0.05 kg/m^3) allowed individuals to move in an uninhibited manner within the enclosure. Although the extent of enclosure-induced behavioral modification cannot be quantified in our study, the ability of our simple linear model to predict accurately the NFS for a noncaptive school is very encouraging.

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Abstract—We examined the effects of gear selectivity and the consequences of a chosen method for estimating growth parameters of bluefish, *Pomatomus saltatrix*, from southern Brazil. Samples were obtained from commercial landings at Rio Grande (1992–97). Age determination for 1159 fish indicated that gill-net and purse-seine fisheries caught 1–5 year-old and 1–7 year-old fish, respectively. Trawlers caught fish from ages 1 to 7. Because of their high degree of selectivity, the gill nets caught the larger individuals of ages 1 and 2, as well as the smaller individuals of ages 3–5. Faster-growing fish captured with gill nets had smaller scales than fish of a similar length caught with purse seines. By contrast, the slower-growing fish captured with gill nets had larger scale size than fish of a similar length caught with purse seines. For gill nets and purse seines, there were differences between the von Bertalanffy growth estimates derived from both mean values of back-calculated length-at-age and individual back-calculated length-at-age. We also recorded differences in growth parameters obtained from back-calculated length-at-age derived from measurements to the last annulus only and from measurements of all annuli up to the sampling age. Selectivity-related bias was incorporated in the estimation of growth parameters, yielding unrealistic estimates of L_∞ and k for the gill-net growth curve.

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Effects of gear selectivity and different calculation methods on estimating growth parameters of bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae), from southern Brazil

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The bluefish, *Pomatomus saltatrix* (L.) (Pomatomidae), is a highly mobile pelagic predator (Haimovici and Krug, 1996), widely distributed along the continental shelf in the temperate and warm waters of the Atlantic, Pacific, and Indian oceans (Wilk, 1977). Abundant in Brazilian waters, this species is commercially exploited by purse-seine and gill-net fleets operating in the subtropical coastal waters of Rio Grande do Sul between Conceição (31°42′S) and Chuí (33°43′S), at depths between 8 and 100 m (Lucena and Reis, 1998) (Fig. 1). Purse-seine boats are approximately 23 m in length and have 220–330 HP engines, whereas gill-net boats are usually 14–16 m in length and have 90–150 HP engines (Boffo and Reis, 1997). Gill nets used in the bluefish fishery are drift gill nets 1800 m in length and that have a stretched mesh size of 90 mm.

Commercial catches of bluefish were first monitored from 1976 to 1983 on the fishing grounds from Sarita (32°37′S) to Conceição at depths up to 35 m (Krug and Haimovici, 1991). Bluefish were then primarily exploited by purse seine but were also taken as bycatch in a gill-net fishery targeting wreckfish (*Polyprion americanus*) and elasmobranchs. Commercial catches were dominated by fish between 1 and 3 years old for the purse-seine fleet and by fish aged 2–4 years old for the gill-net fleet (Krug and Haimovici, 1989).

As catches from the estuarine Patos Lagoon declined, beginning in 1982, the artisanal fleet began exploiting new fishing grounds and a fishery developed in shallow coastal waters (Reis, 1992). Since 1990, bluefish have been caught primarily by gill net (up to 72% of landings by weight) and the fishing grounds now extend southward into waters up to 100 m (Lucena and Reis, 1998). For the period 1991–95, commercial catches have included individuals to age 10 (Lucena, 1997).

Since the emergence of the gill-net fleet, the exploitation of the bluefish has been increasing. From 1991 to 1996, there has been an 87% increase in the length of netting deployed and a 166% increase in the soak time of the gear (Lucena and Reis, 1998). In addition, there has been a decrease in mean length of fish caught by purse seines and gill nets from 1992 to 1995 (Lucena, 1997). Average annual landings of the bluefish decreased from 3100 (during the period 1990–95) to 1700 tonnes (during 1996–98) (IBAMA[1]).

The growth parameters of the southern Brazilian bluefish population were estimated by Krug and Haimovici (1989), using scales from fish landed commercially by several gears (gill nets, purse seines, trawlers, long lines, and beach netting) from 1976 to 1983. Their study excluded individuals larger than 63 cm TL because the larger fish lived at depths greater than 35 m, the operational limit of the fleet at that time. Given today's increased exploitation of bluefish, and the lack of older fish in the earlier study, we decided to examine the age structure and growth parameters of the bluefish stock off southern Brazil. New data are compared to previously published information and the effects of gear selectivity on the estimation of growth parameters are also evaluated.

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[1] IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renovaveis). 1990–1998. Unpubl. data. Centro de Pesquisa do Rio Grande (CEPERG)–RS, IBAMA, Brazil. Cep: 96201-900.

Annulus formation occurs in winter (June to September) and is associated with lower water temperature (Haimovici and Krug, 1996). Rings do not become visible until growth resumes in January or February. During peak spawning, January–February, the true age of individuals with one ring is 7 months on average (Haimovici and Krug, 1992).

Even though most sampled bluefish have usually been caught during the short period of the fishing season (June to September), the real age of a fish may vary by between 4 and 9 months from that notionally adopted because the bluefish is a multiple spawner and because this species' scale ring formation is associated with lower temperatures (the exact timing of ring formation varies from year to year and can also be influenced by environmental phenomena such as El Niño events). Hence, because sample collections were spaced unevenly throughout the fishing season for both within-year and between-period comparisons, growth rates based on changes in observed lengths were deemed inappropriate and the estimation of growth parameters was based on back-calculated lengths.

Many early writers, using the back-calculation technique, made no mention of the reason for choosing a particular procedure (e.g. Johnson and Saloman, 1984; Barger, 1990; Vieira and Haimovici, 1993). Valuable reviews on back-calculation were carried out by Hile (1970), Tesch (1971), Casselman (1987) and Francis (1990) and, although this technique is widespread, it still does not appear to be well understood. Moreover, there is a common tendency to plot only mean, rather than individual, back-calculated length-at-age to produce the growth curve (Hilborn and Walters, 1992). The effects of different approaches on growth parameter estimates are discussed in this paper.

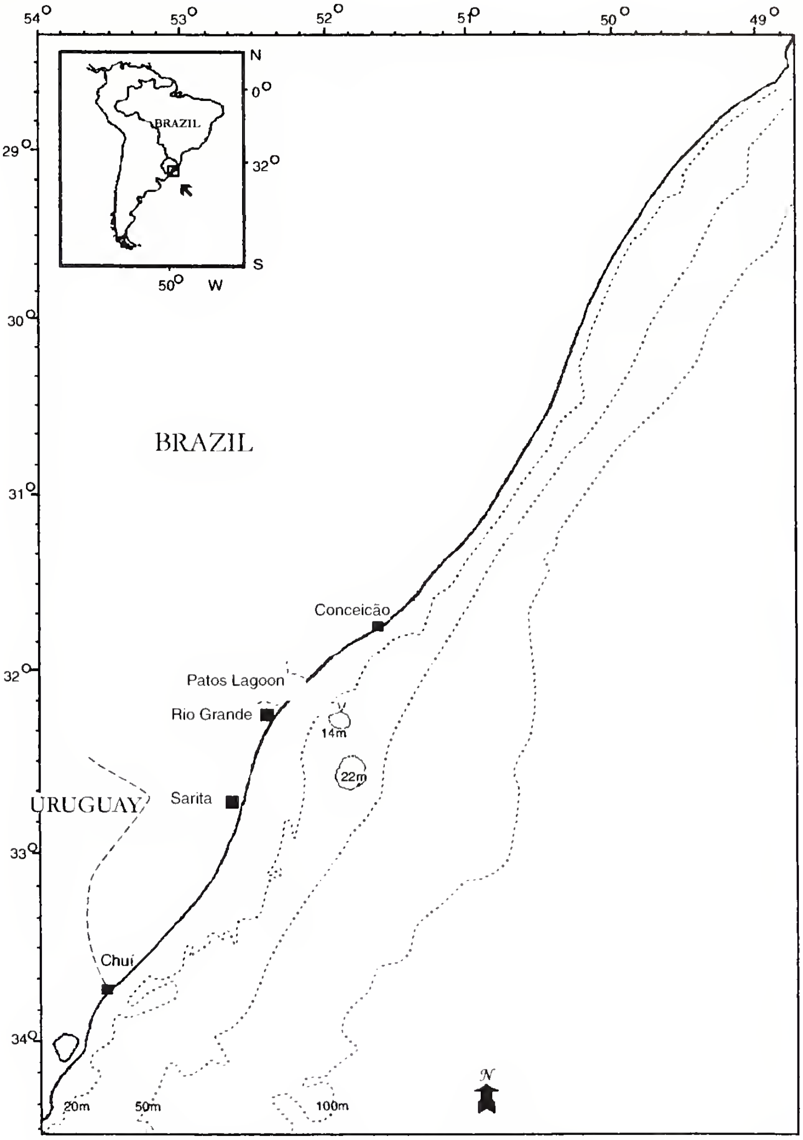

Figure 1

Map of the southern coast of Brazil and sampling area for bluefish.

Materials and methods

Sampling, age structure, and scale reading

We sampled commercial landings from three fleets at Rio Grande from 1992 to 1997: catch from the gill-net fleet, catch from the purse-seine fleet, and bycatch from the trawl (pair and otter) fleet (Table 1). We sampled 13 additional bluefish from the gill-net fishery targeting wreckfish (*Polyprion americanus*), choosing larger (older) fish in order to investigate the size of the bluefish that move along the shelf break at depths >200 m, where the wreckfish fishery operates. We collected an additional 57 individuals (75–135 mm in TL) with experimental trawls in the estuary of Patos Lagoon in order to calibrate the weight-length relationship for juveniles.

Total length (TL) in mm of 1159 fish (260 to 711 in TL), measured to the end of the extended tail were recorded. The total weight (TW, in grams) and sex (macroscopically determined) of 580 bluefish, ranging in size from 75 to 711 mm TL, were determined to calculate the weight-length relationship, by using nonlinear least-squares regression

Table 1

Depth, sampling period, length and age range, and number of boats and individuals examined as part of the bluefish sampling in southern Brazil during the 1992–97 period.

	Bluefish target species		Bluefish bycatch	
	Gill net	Purse seine	Gill net	Trawl (pair and otter) net
Depth (m)	10–35	8–100	270	8–68
Sampling period	Jun–Aug	May–Sep	Sep	Jan–Mar, May
Length range (mm)	301–602	265–711	650–770	262–711
Age range (years)	1–5	1–7	5–10	1–7
Number of boats examined	33	11	1	7
Number of individuals examined	853	233	13	60

(SPSS, 1998). *T*-tests were used to test for gear- and sex-specific length-weight relationships.

Scales of captured bluefish were removed from under the pectoral fin and stored in a coin envelop. The scales of 1159 fish were mounted between glass slides and fish age was determined by following Krug and Haimovici (1989). Fish were considered to be 1 year old after the formation of the first winter ring and a further year was added to the age of the fish for each subsequent ring (Krug and Haimovici, 1989).

Two different readers assigned ages for the fish, with no knowledge of fish length or month and gear of capture. If two readings agreed, then the age of that reading was adopted as definitive. If not, the scale was reread after two months, concurrently by both readers. If two of the three readings were the same, the age of these two readings was assumed as definitive. If all three readings differed, the scale was omitted from further analysis. On some scales, a single reader assigned ages. This method was followed with a time lag (2 months) between readings.

Estimation of growth parameters

Sample sizes were sufficient for estimation of growth parameters from gill-net and purse-seine samples. Ages from the direct reading of scales were obtained for 816 bluefish from gill-net samples and for 212 bluefish from purse-seine samples.

Ricker (1992) pointed out that the inclusion of sampled fish taken at different times during the growing season should be avoided and that preferably fish collected between the formation of an annulus and the start of the next year's growth should be used in analysis. Only data obtained during the fishing season were included for estimation of growth parameters derived from direct reading, and data derived from purse-seine samples (the less selective gear) were used to compare the growth parameters derived from mean observed lengths-at-age and back-calculated lengths-at-age.

For back-calculation of growth parameters, 283 specimens (174 from gill-net samples and 109 from purse-seine samples) were used. Fish were processed by sex and gears used in their capture to avoid bias in results.

We determined the relationship between fish length (TL) and scale radius (S). Krug and Haimovici (1989) previously identified a nonlinear relationship between fish length and scale radius for the southern Brazilian bluefish ($TL=aS^b$). Back-calculated total lengths at age were determined by using the formula of Monastyrsky (see Bagenal and Tesch, 1978; Francis, 1990):

$$L_n = (S_n / S)^b \times TL,$$

where L_n = the length of fish when annulus "n" was formed;
S_n = the radius of annulus "n" (at fish length L_n); and
b = the slope from the body-scale relationship TL on S.

This nonlinear method for back-calculation assumes that the relationship TL on S is of the form $TL = aS,^b$ where a and b are parameters to be estimated usually by nonlinear least squares.

The periodicity of growth increments was examined by using analysis of marginal increments $[(S - S_n)/(Sn - S_{n-1})]$ from fish 2–7 years old (mainly 2–3 years old) (following Krug and Haimovici, 1989). Because of the limited temporal range of samples, no marginal increments analysis was done for the months March–April and November–December.

We derived theoretical growth parameters by fitting back-calculated lengths-at-age to the von Bertalanffy (1934) growth equation

$$L_t = L_\infty\left(1 - \exp^{-k(t-t_0)}\right),$$

where L_t = the length at age t;
L_∞ = the asymptotic length;
k = the brody growth coefficient; and
t_0 = the age when length would theoretically be zero.

Estimation of growth parameters was based upon two criteria. Criterion I identified the type of summary statistic to be used:

Ia corresponded to mean values of back-calculated lengths-at-age; and
Ib represented individual back-calculated lengths-at-age.

Criterion II identified the type of data to be used:

IIa corresponded to the back-calculated length-at-age derived from the last annulus only; and
IIb represented the back-calculated lengths-at-age derived from all the annuli up to the sampling age.

We calculated growth parameters by using four methods for each gear: method 1 (where criteria Ia and IIa were used), method 2 (where criteria Ia and IIb were used), method 3 (where criteria Ib and IIa were used), and method 4 (where criteria Ib and IIb were used).

The growth curves were estimated with the program AD Model Builder (Otter Research, 1996), which uses an automatic differentiation algorithm to estimate the parameters of a nonlinear function with an appropriate objective function. The software allows the calculation of confidence intervals for parameter estimates with asymptotic normal approximations.

The objective function considered is the nonlinear least-squares regression criterion, $R(.)$, either given by

$$R\left(L_\infty,k,t_0 \middle| O_t\right)=\sum_{t=1}^{T}(O_t-L_t)^2,$$

when mean values are considered, or given by

$$R\left(L_\infty,k,t_0 \middle| O_{ti}\right)=\sum_{t=1}^{T}\sum_{i=1}^{n_t}(O_{ti}-L_t)^2,$$

when individual values are considered.

Note that O_t is the mean back-calculated length-at-age t, O_{ti} is the ith individual back-calculated length-at-age t, T denotes the maximum number of distinct ages, and n_t is the number of observations at age t. To compare growth curves between gears, Hotelling's t-test (1979) was used.

Results

Age structure of the stock

We examined a total of 1159 fish and 94% of samples were collected during the fishing season (June to September)—the remaining numbers represented bycatch during the off-peak season. Age determination (Fig. 2) indicated that the gill-net fisheries caught fish of ages 1–5 (mode=3 yr) and purse-seine fisheries caught fish of ages 1–7 (mode=2–3 yr). Trawlers caught fish of ages 1–7. Individuals of ages 5 to 7 represented less than 2% of samples (individuals of 5 to 7 years old were underrepresented also in the back-calculation procedures). The deep-water (to 200 m) gill-net fishery targeting *P. americanus* caught individuals of age 5–10 and the only fish older than age 7. There was a considerable overlap in the range of observed lengths-at-age (Table 2), nevertheless significant differences between gears were detected for some ages. Gill nets caught larger individuals of ages 1 and 2 and smaller individuals of ages 3–5 than purse seines. Trawls, on the other hand, appear to catch smaller individuals in all age classes.

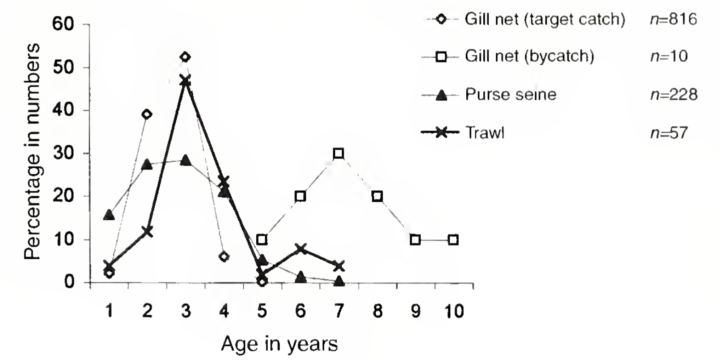

Figure 2

Relative proportion (%) of bluefish caught by various fisheries operating off Brazil's southern coast.

Weight-length relationship

We found no statistically significant differences in length-weight relationships between gear types or between sexes (t-test, $P>0.05$). The relationship for both sexes and all gears combined is illustrated in Figure 3.

Body-scale relationship

Gear-specific regressions of TL on S (Fig. 4) showed that gill nets tended to catch the fast-growing fish of younger ages and the slow-growing individuals of older ages. These faster-growing younger fish (<360 mm TL, 1–2 years) have smaller scales than similar-size purse-seine–caught fish (t-test; $P<0.05$). By contrast, the slower-growing fish (>410 mm TL, 4–5 yr) had larger scales than fish of a similar length caught by the purse-seine fleet. For the length range that the gill nets target most efficiently (360–410 mm TL, age 3), the TL-S relationship for both gill net and purse seine was similar.

Periodicity of growth increments

Our limited data showed marginal increment to be lowest in January–February, corresponding to ring deposition.

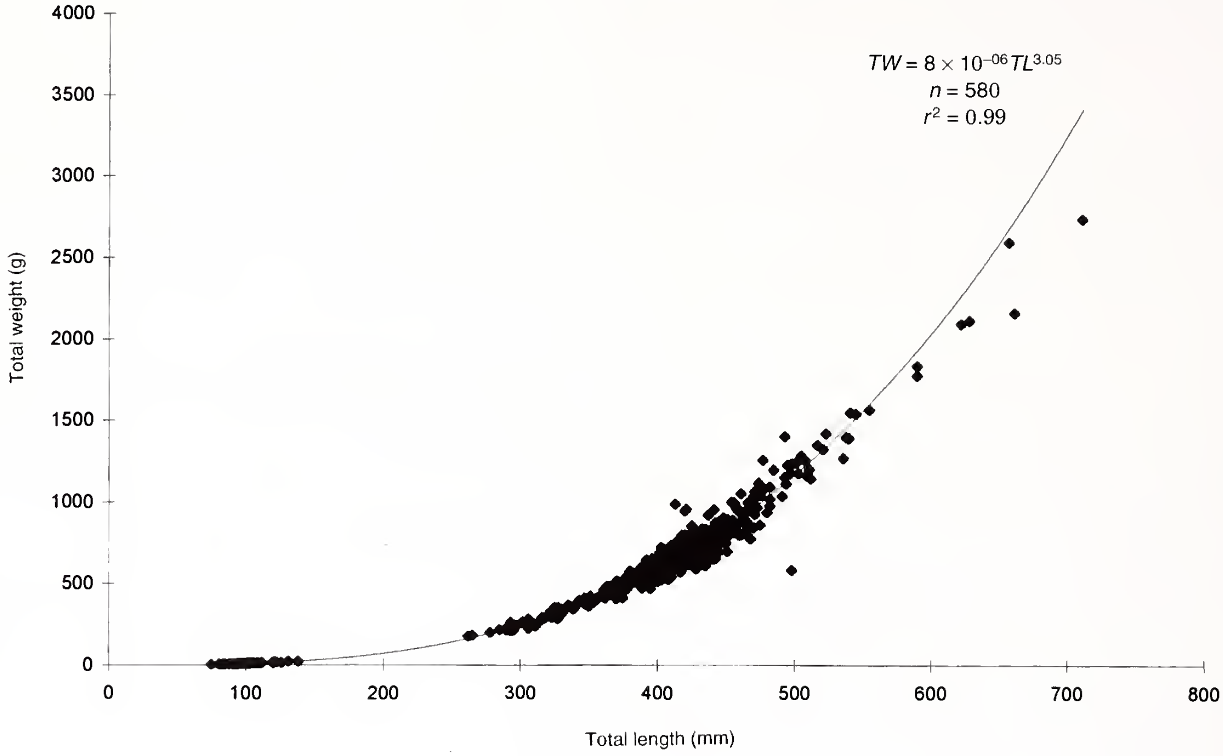

Figure 3

Weight-length relationship for bluefish (sexes and gears combined).

Table 2

Mean observed length-at-age for gill-net (targeted catch), purse-seine, and trawl catches. The symbol SD denotes the standard deviation, n denotes the number of individuals examined and "—" denotes that a value could not be calculated. ANOVA was performed only for the age classes with n greater than 10.

Age (yr)	Gill net length at age (mm)			Purse-seine length at age (mm)			Trawl length at age (mm)		
	Average	Range	SD (n)	Average	Range	SD (n)	Average	Range	SD (n)
1	354.3[1,2]	301–417	30.8 (33)	313.5[1]	276–385	23.4 (31)	278.5	265–292	19.0 (2)
2	408.7[1]	293–495	32.6 (286)	385.0[1]	290–485	44.5 (49)	372.8	285–409	47.0 (6)
3	438.9[1]	328–543	31.0 (431)	460.4[1,3]	364–520	40.7 (61)	419.4[3]	361–498	30.6 (24)
4	467.7[1]	410–602	42.3 (65)	518.1[1,3]	451–622	38.3 (62)	451.5[3]	342–554	54.7 (12)
5	498.0		— (1)	552.5	418–711	54.4 (17)	474	—	— (1)
6				622.7	556–661	38.7 (7)	507	477–661	89.3 (4)
7				664.5	657–672	10.6 (2)	651	642–660	12.72 (2)

[1] Significant difference between lengths-at-age for gill-net and purse-seine catches (ANOVA, $P<0.05$).

[2] Significant difference between lengths-at-age for gill-net and trawl catches (ANOVA, $P<0.05$).

[3] Significant differences between lengths-at-age for purse-seine and trawl catches (ANOVA, $P<0.05$).

Table 3

Mean back-calculated total lengths (mm) of bluefish for the purse-seine and gill-net fishery. *Lc* = Mean observed lengths-at-age. Mean back-calculated lengths-at-age derived from the last annulus are shown in bold.

Age-group	*Lc*	*n*	SD (last annulus only)	Method 1	2	3	4	5	6	7
Purse-seine fishery										
1	313.5	22	27	**202.2**						
2	385.0	26	31	189.0	**315.5**					
3	460.4	23	36	227.9	358.0	**437.3**				
4	518.1	26	35	234.5	373.1	462.4	**504.3**			
5	552.5	8	72	225.4	366.9	455.4	504.5	**547.1**		
6	622.7	3	30	219.1	352.5	447.2	502.0	552.7	**607.9**	
7	664.5	1	—	247.9	352.6	512.1	574.6	615.9	636.5	**647.4**
Mean (all annuli)		109		214.6	350.6	451.3	506.1	554.4	615.4	647.4
SD				36	47	45	46	64	28	—
Gill-net fishery										
1	354.3	18	27	**236.2**						
2	408.7	59	46	230.4	**346.1**					
3	438.9	75	30	239.8	358.2	**430.3**				
4	467.7	21	41	244.9	366.2	445.5	**480.8**			
5	498.0	1	—	196.3	350.3	403.7	426.5	**460.0**		
Mean (all annuli)		174		236.7	354.9	433.3	483.4	460.0		
SD				41	39	58	41	—		

Growth occurs from May to October and peaks in August. From these data, we believe that the growth increments are annuli.

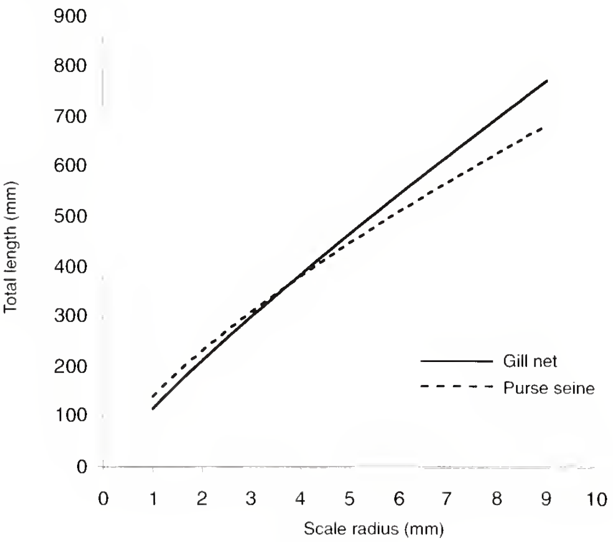

Figure 4

Total length-scale relationship derived from gill-net samples ($TL=140.5S^{0.72}$, $n=174$) and purse-seine samples ($TL=116.7S^{0.86}$, $n=109$).

Back-calculated length-at-age and estimation of growth parameters

Back-calculated lengths-at-age calculated by using the last annulus only (IIa) were smaller than lengths-at-age calculated by using all annuli (IIb) (Table 3). There were also significant differences in lengths-at-age for these two criteria between gear types (*t*-test, $P<0.05$ for all age classes). Sexes were combined because there was no significant difference between them (*t*-test, $P<0.05$). Back-calculated lengths-at-age for gill-net samples showed a decreasing pattern for older fish (5 years old), indicating the presence of Lee's phenomenon and the selective effects of this gear.

Estimates of theoretical growth parameters indicated differences by gear and method of estimation (Table 4, Fig. 5). For criterion I, estimates with mean back-calculated lengths-at-age (criterion Ia) produced higher values of L_∞ (and smaller values of k) for purse-seine samples than estimates with individual back-calculated lengths-at-age (criterion Ib). The opposite trend was observed for the gill-net samples.

On the other hand, for criterion II, especially for purse-seine samples, estimates of mean back-calculated lengths-at-age derived from the last annulus only (criterion IIa) produced smaller values of L_∞ (and higher values of k) than estimates with mean back-calculated lengths-at-age for all annuli (criterion IIb) (Table 4). Furthermore, for the purse seine, the growth parameters derived from methods 3 (Ib, IIa) and 4 (Ib, IIb) were distinct (Table 4). The at-

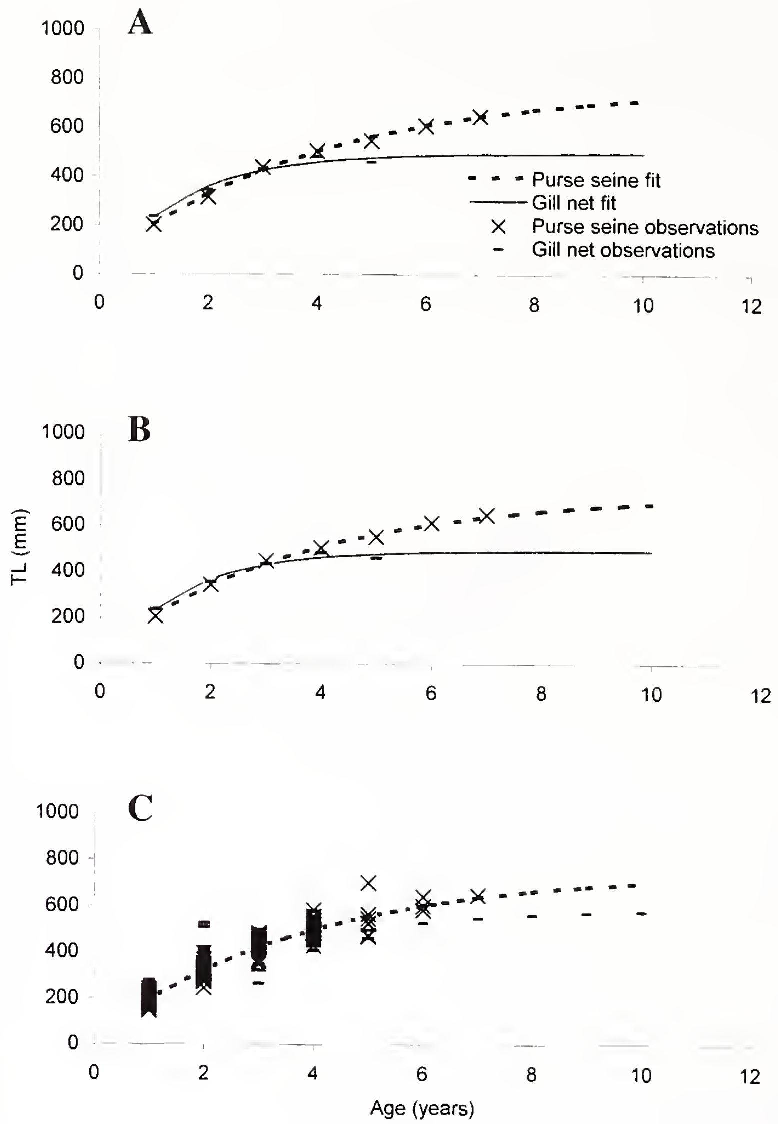

Figure 5

Growth curves for bluefish caught by purse seines and gill nets calculated by (**A**) method 1 (mean lengths—last annulus only) (**B**) method 2 (mean lengths—all annuli) and (**C**) method 3 (individual lengths—last annulus only).

tempt to fit the von Bertalanffy growth curve by method 4 for the gill net failed because no feasible solution could be found for the parameters of the curve.

For all methods there were significant differences in growth parameter estimates between the gill-net and the purse-seine samples (Hotelling's t-test, $P<0.05$).

The comparison between mean back-calculated (especially criterion IIa) and mean observed length-at-age showed that the latter is systematically larger than the former (Table 3). This difference was expected because the mean back-calculated lengths-at-age were obtained for length at time of annulus formation (where spawning season and time of annulus formation are considered uniform for all fish). The differences between both means may be attributed to growth after the growth mark formation. Moreover, the mean observed length-at-age calculated from sampled fish was obtained from individuals that may have spawned at different times of the year or whose ring formation may have been distinctly visible in time, resulting in fish with distinct real ages. Hence, growth parameters obtained from mean observed lengths-at-age of purse-seine catches during the fishing season (L_∞=985 mm, k=0.12 and t_0= 2.17) were significantly different from the growth parameters derived from back-calculated lengths-at-age (also from purse-seine catches) (Hotelling's t-test, $P<0.001$ for all methods).

Table 4

Growth parameters and correlation between k and L_∞ for different methods (1–4), and respective criteria (Ia, Ib, IIa, IIb) of back-calculation. Standard errors are given in parenthesis. "—" denotes that parameter estimates did not converge to a solution.

	Purse seine				Gill net			
Parameters	[1] Ia–IIa	[2] Ia–IIb	[3] Ib–IIa	[4] Ib–IIb	[1] Ia–IIa	[2] Ia–IIb	[3] Ib–IIa	[4] Ib–IIb
L_∞	773 (32.6)	743 (26.6)	754 (53.8)	670 (55.2)	496 (21.3)	491 (17.1)	589 (45.3)	—
k	0.25 (0.03)	0.27 (0.03)	0.26 (0.04)	0.35 (0.07)	0.65 (0.14)	0.71 (0.13)	0.39 (0.08)	—
t_0	−0.21 (0.12)	−0.27 (0.12)	−0.15 (0.12)	−0.09 (0.13)	0.03 (0.20)	0.09 (0.17)	0.27 (0.2)	—
$r\ (k, L_\infty)$	−0.97	−0.97	−0.98	−0.98	−0.89	−0.87	−0.98	—

Discussion

Back-calculation of length is widely used to obtain growth curves, to estimate length-at-age of individuals that are rarely observed, to compare growth differences among populations or sexes of the same species, and even to illustrate gear selectivity (Francis, 1990). However, this technique is poorly understood (Francis, 1990; Rijnsdorp et al., 1990) and some of the sources of bias in using the technique are 1) inaccurate counts of annuli and incorrect estimation of time of formation of the growth mark; and 2) an erroneous choice of the mathematical function to describe the body-scale relationship. In respect to the first source of bias, our results corroborate the findings of Krug and Haimovici (1989). In respect to the body-scale relationship, the main concern of Francis (1990) was to decide whether the appropriate regression was *TL* on *S* (body proportional) or *S* on *TL* (scale proportional). The small difference between the back-calculation derived from the two approaches for the bluefish can be a minimum measure of precision of the back-calculation procedure (Francis, 1990). Campana (1990) and Wright et al. (1990) pointed out that slow-growing fish have larger bony structures (scales or otoliths) than fast-growing fish of the same size. For the bluefish body-scale relationship, we attribute this effect to gear selectivity. The fast-growing fish (ages 1 and 2 from the gill-net catches) tend to occur above the purse-seine curve and the slow-growing fish (ages 4 and 5 from the gill-net catches) tend to occur below the purse-seine curve in a TL-S relationship.

An additional source of bias may be introduced depending on whether mean back-calculated lengths-at-ages or individual lengths-at-age are used to fit the von Bertalanffy equation. The estimated growth parameters derived from the two methods may differ considerably (Hilborn and Walters, 1992). Use of mean values would ignore individual variability in length-at-age, giving the same weight for possibly uncertain ages because of low sample sizes.

We suggest that back-calculated lengths-at-age derived from the last annulus only (criterion IIa) be used rather than the back-calculated lengths-at-age derived from all annuli (criterion IIb). Many investigators continue to use mean-weighted data (all annuli) (e.g. Krug and Haimovici, 1989; Barger, 1990)—a procedure in violation of the least-squares assumption of independence of sample elements (Draper and Smith, 1966). This assumption is violated when multiple measures from a single fish are used. Use of the last annulus only does not use all information available about growth of all cohorts and may result in an incorrect estimate of lengths-at-age for age classes absent or infrequently caught. However, if representative samples are available for younger age groups, the use of back-calculated lengths-at-age derived from the last annulus only is to be preferred.

Ricker (1969) noted that where differential mortality exists, the calculated average length of a particular age class calculated from the last annulus differs from the calculated average length of an age class calculated from previous annuli—the latter representing the former size of the fish that has survived to the sampling age. The differential mortality may be due to natural or fishing causes but under these circumstances, the average size of fish in the year class becomes different as time passes and the frequency distribution of the survivors would become progressively skewed. Gutreuter (1987) suggested that back-calculation should be restricted to the most recent annulus to avoid bias from size-selective sampling.

Gear selectivity can influence estimates of growth parameters (Ricker, 1969; Potts et al., 1998), and we found that gear-related differences in parameters were significant. Gill nets, on the basis of their mesh size, are a selective gear. Trawls are selective for smaller fish that may not be able to avoid the nets because of slower swimming speed (Hilborn and Walters, 1992). Purse seines are probably a less selective gear (Cushing, 1968). Observed lengths-at-age reflect the differences in selectivity between gears. Gill nets catch the faster-growing fish at age 1 and 2 and the slower-growing fish at age 4 and 5, and trawls catch the smaller individuals for each age.

The high selectivity of gill nets is visible in the body-scale relationship, the mean observed length-at-age, and in the back-calculated lengths-at-age which indicate the presence of Lee's phenomenon (Lee, 1920). Size-selective mortality—caused by the differing catchabilities of fish at different sizes—is the probable reason for Lee's phenomenon in back-calculation of lengths from gill-net catches.

Table 5

Back-calculated length-at-age and growth parameters obtained by various authors and at a number of locations. Probability values calculated from tests between means ($P<0.05$) for the bluefish in southern Brazil (our study and published studies) are given in parentheses. Mean back-calculated length-at-age derived from all annuli was used to allow comparison between studies.

Location	Age class									k	L_∞	Source
	1	2	3	4	5	6	7	8	9			
Black Sea	212	322	409	562	615	663				0.150	1080	Kolarov (1963)
Senegal	209	375	500	604	689	758	913	858	893	0.018	1044	Champagnat (1983)
Long Island, USA	230	400	490	580	640	690	710			0.340	795	Richards (1976)[1,2]
Mexico	308	413	509	576	627	675	715	766		0.180	944	Barger(1990)[1]
West Atlantic	290	361	415	473						0.096	1019	Barger (1990)[1]
South Africa	165	279	369	437	480					0.196	840	Van de Elst (1976)
Southern Brazil	196	356	438	506	562	600	618			0.380	662	Krug and Haimovici (1989)
Southern Brazil	214	351	451	506	554	615	647			0.260	754	Our study
	(P=0.0)	(P=0.21)	(P=0.03)	(P=0.99)	(P=0.57)	(P=0.39)	—[3]					

[1] Fork length was considered.

[2] Growth parameters were calculated from published mean back-calculated length-at-age.

[3] Sample size was 1.

Selectivity-related bias from gill-net back-calculation is thus incorporated in the growth parameter estimation. The growth curve calculated from gill-net back-calculation reports unrealistic L_∞ and k. Method 4 (individual back-calculated lengths-at-age derived from all annuli) for the gill net failed because no feasible solution could be found for the parameters of the curve. High variability of back-calculated lengths-at-age derived from all annuli (see standard deviations in Table 3) in the presence of Lee's phenomenon may have been the reason for the unsuccessful attempt to obtain estimates of the parameters of the von Bertalanffy growth curve with this method.

In our study, the growth parameters estimated from individual back-calculated length-at-age derived from the last annulus only (method 3) on purse-seine samples were considered to be the most appropriate for future assessment of the southern Brazilian bluefish stock. This gear is less selective than others and data comprise a wider range of total length groups and age groups.

Changes in Brazilian commercial fishing operations for the bluefish over the last decade have led to changes in the length and age structure of commercial landings. From 1976 to 1983 the maximum age and total length of bluefish in the fishery were 7 years and 630 mm TL, respectively, and less than 5% of fish were 4 years and older (Krug and Haimovici, 1989). Currently the fishery lands fish up to age 10, and 16% of fish are aged 4 and older. The inclusion of older individuals in recent catches is due to the expansion of the fishery into deeper areas. In 1990, the purse-seine fishery began moving to deeper waters, catching a wider range of sizes of bluefish. Hilborn and Walters (1992) have pointed out the necessity to understand fleet dynamics in order to assess the population dynamics of a species.

Our mean back-calculated lengths-at-age (calculated from the last annulus only) are similar to those found by Krug and Haimovici (1989) (except for ages 1 and 3) (Table 5), but estimated growth parameters for the two studies are different. We believe these differences are due to differences in fishing operation between the two periods (fleet operated in shallower water in 1977–83 than in 1992–97) rather than to fishing pressure. Also, the use of individual back-calculated lengths-at-age for the last annulus only (method 3) rather than the mean back-calculated lengths-at-age for all annuli (weighted mean, method 2, Krug and Haimovici, 1989) could have led to such a difference.

Data from the literature indicate considerable variation in length-at–age of bluefish between areas (Table 5). Terceiro and Ross (1993) attributed this variation to a sampling bias in available fish and to the differential proportion of spawned fishes in collected samples (e.g. due to multiple spawning). Champagnat (1983) suggested that differences in size at first maturity could lead to differences in growth parameters. The reported size at first maturity of bluefish varies from 250 mm TL in South Africa (Van der Elst, 1976) to 430 mm TL in Senegal (Conand, 1975, Champagnat, 1983). In southern Brazil, length at first maturity for bluefish is between 350 to 400 mm TL (Haimovici and Krug, 1992) and this stock is considered to be relatively fast growing.

It is difficult to obtain samples representative of the population's age structure when using a single gear because of the size selectivity of most gears (Bagenal and Tesch, 1978). Also, the ranges of fish length and age represented in a study may affect the estimation of population parameters if they are not representative of the stock (Goodyear, 1995). Our study suggests that the data used for the estimation of the growth parameters for a fish stock should allow for the possible effects of gear selectivity. Data from different gears should be analyzed and should cover as wide an area of the stock distribution as possible. Moreover, the technique applied for estimation of growth parameters should be carefully assessed and the one chosen, strongly justified. Verification of the validity of the back-calculation method and validation of the annual nature of increment formation are also strongly recommended.

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Abstract—Ladyfish, *Elops saurus*, are recognized as an estuarine-dependent species, although no published study has described how ladyfish use estuarine habitats. This study found ladyfish to be common throughout Tampa Bay and Indian River Lagoon, Florida. In both estuaries, metamorphosing larvae were collected during several months of the year, but they were most abundant in spring. Length-frequency analyses suggested that age-0 ladyfish grew from 20–30 mm to 200–300 mm standard length during their first year and that at least three age classes were present throughout the year. Age-0 ladyfish followed an ontogenetic migration with regard to salinity. They entered estuaries as metamorphosing larvae and became concentrated in waters of lower than median salinity for both estuaries (23–25 ppt). In Tampa Bay, which had a greater range of salinity than the Indian River Lagoon, age-0 ladyfish were found principally in mesohaline and oligohaline areas; in the Indian River Lagoon, age-0 ladyfish were found in mesohaline and polyhaline waters. In autumn, age-0 ladyfish moved back to higher salinities, into lower parts of the estuaries, and even out to beaches along the Gulf of Mexico. These field observations are consistent with the hypothesis that ladyfish depend on estuaries, specifically positive estuaries, i.e. where freshwater input exceeds evaporative processes. However, published studies also demonstrate that larval ladyfish can metamorphose and juveniles can survive in hypersaline waters; therefore negative estuaries may also serve as suitable nursery habitat. It is not clear how salinity affects ladyfish growth and mortality, and further research should clarify how different types of estuaries (i.e. positive versus negative) contribute to maintaining populations of this fishery species.

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Nursery habitats for ladyfish, *Elops saurus*, along salinity gradients in two Florida estuaries

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Many fish species use estuaries of the southeastern United States as nurseries (e.g. Skud and Wilson, 1960; Gunter, 1967), but coastal habitat degradation threatens many of the economically important fisheries that rely on estuaries (Gilmore, 1995). The re-enacted Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) was developed to protect or enhance fisheries habitats, by first requiring information regarding the value of coastal habitats to the survival of marine organisms (e.g. Schmitten, 1996). A simple approach to ranking relative habitat value is to compare intraspecific fish distributions with respect to habitat in different estuaries. Associations between abundance and habitat can assist in predicting the response of coastal fish populations to changes in these habitats. Remarkably, such information is rarely available except for the most economically valuable species (Haedrich, 1983).

Freshwater inflows to estuaries of the southeast United States have been severely altered in the last 150 years and coastal development continues to divert more water away from estuaries (Stickney, 1984). The MSFCMA's Essential Fish Habitat mandate provides a policy framework for identifying the effects of reduced freshwater inflows on estuarine-dependent species. Yet researchers and managers often characterize species as estuarine-dependent more on intuition than rigorous examination of data. Able and Fahay (1998) outlined three criteria for defining estuarine-dependence: 1) predictable use of estuaries, 2) non-use of suitable alternative habitats, and 3) demonstrable effect on a fish population from a loss of estuarine habitat. The first two criteria are best addressed with field studies, but even such simple descriptions of habitat use at a landscape level are lacking for most estuarine fish species (Hoss and Thayer, 1993).

One example of this type of data gap is that for ladyfish, *Elops saurus*, a fishery species that inhabits coastal waters of Florida (Hildebrand, 1963; Murray et al., 1987; FMRI[1]). Ray (1997) listed ladyfish as an estuarine-dependent species but little is known about its use of habitat in coastal waters. Ladyfish are probably considered to be estuarine-dependent because they spawn in offshore waters and metamorphosing larvae and juveniles are found inshore (Hildebrand, 1943; Gehringer, 1959; Eldred and Lyons, 1966). However, ladyfish tolerate a wide range of salinities (Alikunhi and Rao, 1951; Gunter, 1956; Gehringer, 1959; Bayly, 1972). Numerous studies have reported the occurrence of small juveniles in mesohaline or lower-salinity waters (<18 ppt), which is consistent with an estuarine-dependent life history (Tagatz and Dudley, 1961; Gunter and Hall, 1965; Zilberberg, 1966; Tagatz, 1968; Tagatz and Wilkens, 1973; Sekavec, 1974; Govoni and Merriner, 1978 [and references within]; Thompson and Deegan, 1982; Peterson and Ross, 1991). Moreover, large ladyfish are present throughout

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[1] FMRI (Florida Marine Research Institute): www.floridamarine.org

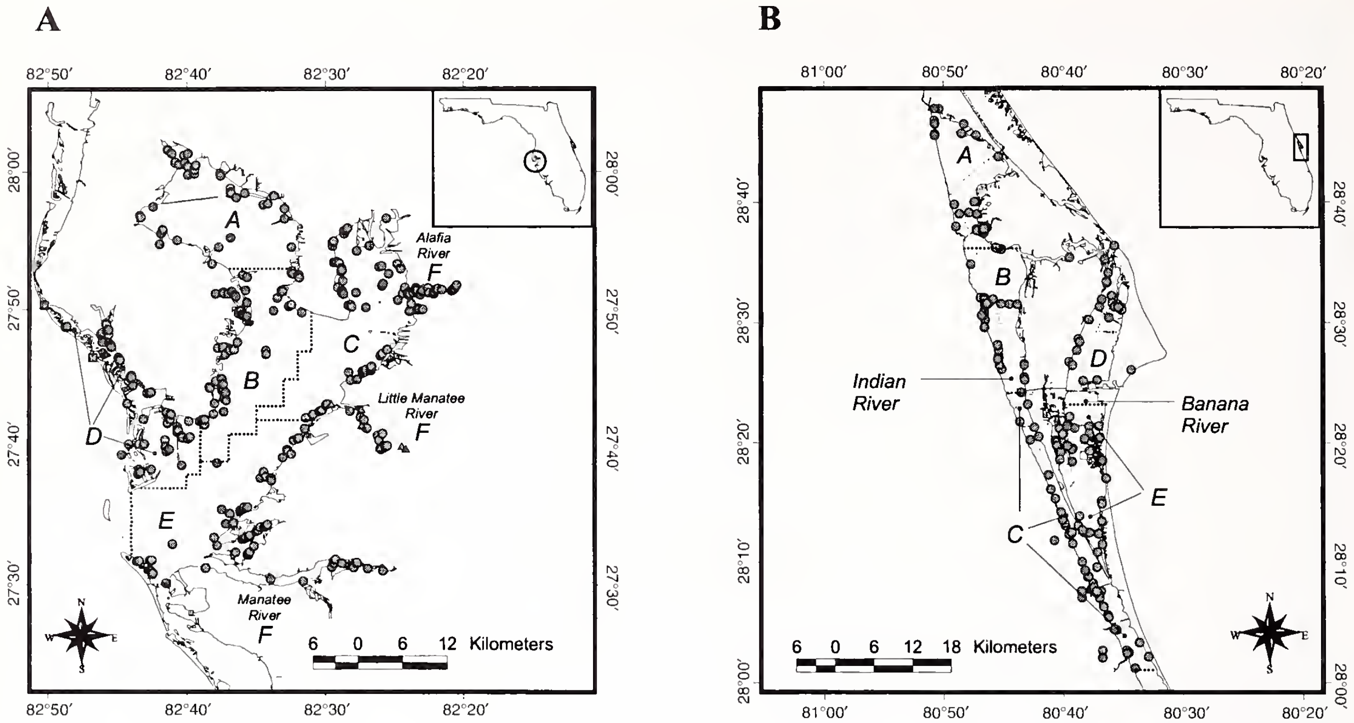

Figure 1

Sampling areas and locations where at least one ladyfish, *Elops saurus*, was collected for (**A**) Tampa Bay and (**B**) the Indian River Lagoon. Different symbols are used to represent collections in Tampa Bay: the bay-wide program (circles), the Little Manatee River program (triangles), and the Gulf Beach program (squares). Lines and letters (A–F) designate the sampling zones in each system. See text for further definition of zones and Tables 1–2 for details of all collections.

the year in polyhaline and marine (>18 ppt) waters such as Florida Bay, Biscayne Bay, and the Indian River Lagoon (Low, 1973; Sogard et al., 1989a, 1989b; Tremain and Adams, 1995). Other studies, however, have reported presumptive age-0 juveniles (Harrington and Harrington, 1961; Kristensen, 1964), older subadults (Carles, 1967), or both (Simmons, 1957; Roessler, 1970; Brockmann, 1974) in hypersaline waters (>35 ppt). Thus, age-0 ladyfish may be seeking some condition other than specific salinities.

The purpose of our study was to determine whether age-0 ladyfish make predictable size-specific seasonal movements that would identify them as an estuarine-dependent species. Because salinity is part of the definition of an estuary (Pritchard, 1967), a landscape approach was used to follow ladyfish movements with respect to salinity within Tampa Bay and the Indian River Lagoon. If age-0 ladyfish select low-salinity waters, then we predicted that they would be found in lower than average salinity waters for each estuary. Furthermore, if age-0 ladyfish select low-salinity habitats, then we predicted that they would be more abundant in the lower salinity areas of Tampa Bay because this bay has a wider salinity range to select than that for the Indian River Lagoon. We also examined length-frequency data to make preliminary assessments of age-class composition and growth rates of ladyfish within estuaries. Although several publications have discussed the ecology of ladyfish in estuaries (e.g. Springer and Woodburn, 1960 and citations above), to our knowledge the data treatment in our study is the most comprehensive for this species.

Methods

Study sites

Data from Tampa Bay, on central Florida's west coast, were compared with data from the Indian River Lagoon, on central Florida's east coast (Fig. 1). Both water systems are located at similar latitudes, so they are subject to similar temperature cycles. Both systems are positive estuaries (i.e. freshwater input exceeds evaporative processes; Pritchard, 1967), but they have distinctive salinity regimes (Fig. 2). Tampa Bay is a drowned river system with considerable freshwater input and separate satellite barrier-island embayments, whereas the Indian River Lagoon is largely a series of barrier-island embayments with few inlets and no major rivers (Comp and Seaman, 1985).

Bay-wide survey—Tampa Bay We examined survey data for Tampa Bay fishes collected from 1989 to 1995 by staff of the Florida Marine Research Institute (FMRI). The sampling design incorporated both fixed sampling stations and locations assigned in a stratified random manner (McMichael et al., 1995). Tampa Bay was stratified into six major zones (Fig. 1A): the upper bay (A), the western bay (B),

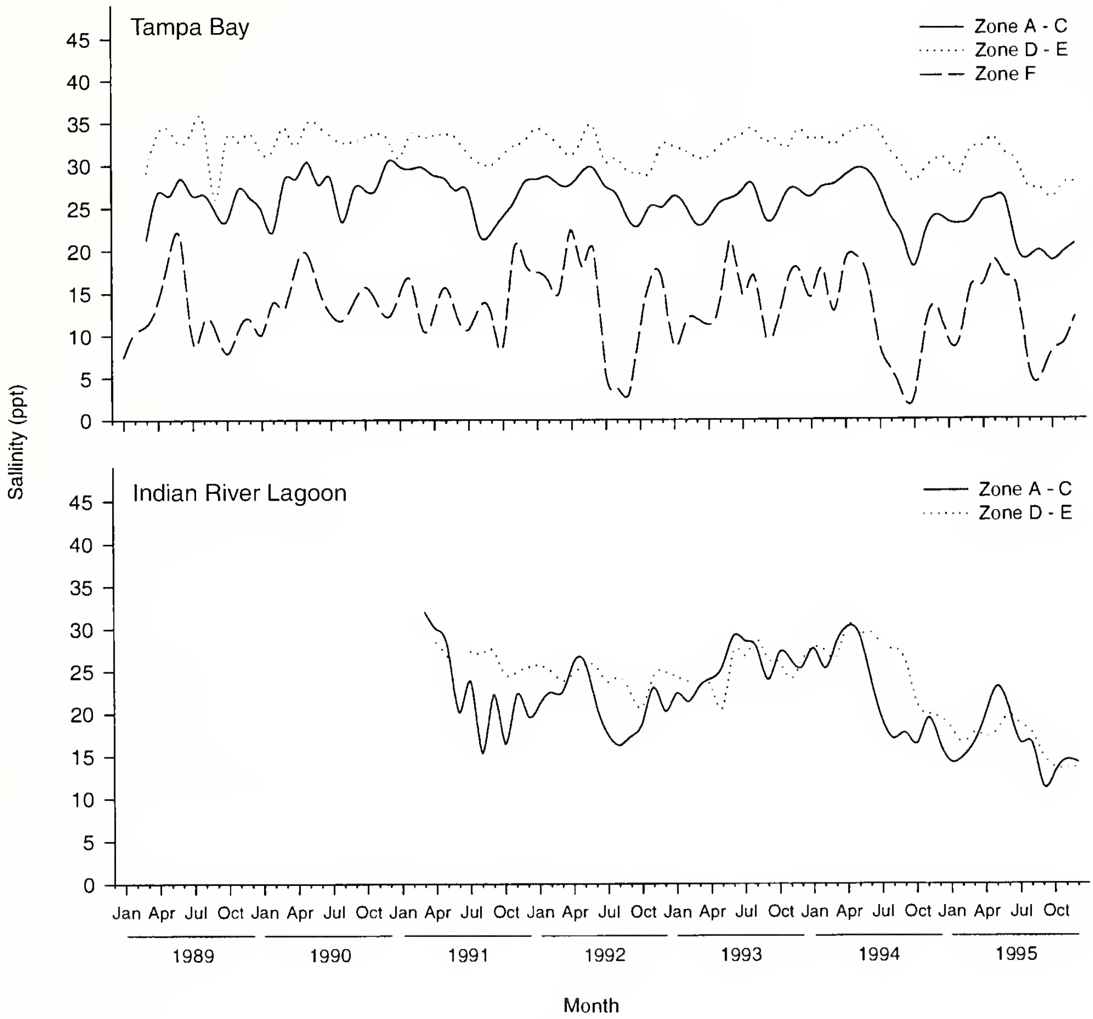

Figure 2

Salinity trends for Tampa Bay (1989–95) and the Indian River Lagoon (1991–95). Mean monthly salinity values were calculated based on all available data and fitted with spline curves. Some sampling zones were combined for graphical simplicity (compare with Fig. 1). Indian River Lagoon 1990 data, which were collected only during the spring (Mar–May) and fall (Sep–Nov) months, were not included.

the eastern bay (C), the satellite embayments (D), the lower bay (E), and the principal rivers (F). Each zone was stratified further by a 1-square-nautical-mile grid system. Sampling at fixed stations occurred monthly, but stratified random sampling was conducted principally in spring and autumn when many species recruit to Florida estuaries.

Sampling gear included seines, trawls, block nets, and gill nets (Table 1). A 21.3-m center-bag seine was deployed in one of three different ways: 1) it was hauled along the shoreline across an area of about 340 m^2 ("beach sets"); 2) it was deployed from a boat in a semicircular pattern in river zones and the mean area swept was 70 m^2 ("boat sets"); 3) or it was set away from the shoreline and hauled into the current across an area of about 140 m^2 ("offshore sets"). A much larger seine (183-m) was also deployed from a boat in a semicircular pattern. A 61-m block net was set against seawalls or mangroves of inundated shorelines at high tide, and fish were collected at the ensuing low tide. Otter trawls were towed for 10 min in bay zones (zones A–E) and for 5 min in river zones (zone F) at an approximate speed of 0.6 m/s. A 184-m gill net with four 46-m panels (75-, 100-, 125-, and 150-mm mesh) and a similar 198-m gill net that included a 15-m section of 50-mm mesh were used. These gill nets were set perpendicular to shore, four nets at a time, so that two nets with the larger mesh were oriented inshore and two nets were oriented in the opposite direction.

Little Manatee River—Tampa Bay In addition to sampling the Little Manatee River as part of the bay-wide survey, FMRI staff completed an independent survey of this river

Table 1

Sources and details of data examined from Tampa Bay: sampling gear used (with mesh size); geographic zones sampled (see Fig. 1A); diel periods (D=day, N=night, C=crepuscular); months (1=January); years sampled during 1989–95; number of ladyfish collected (C (c)[4]); and total number of sets with each net (f). The bay-wide survey of Tampa Bay included a stratified, random survey design and a fixed-station sampling design. The special survey of the Little Manatee River and the Gulf Beach survey used fixed stations.

Gear	Mesh (mm)	Zones	Period	Months	Years	C (c)[4]	f
Bay-wide fixed-station survey							
21.3-m seine (340 m^2)[1]	3.2	A, (B–D),[2] F	D	1–12	1989–95	60	377
21.3-m seine (70 m^2)[1]	3.2	F	D	1–12	1989–95	942 (6)	1818
21.3-m seine (140 m^2)[1]	3.2	A–E	D, N, C[2]	1–12	1989–95	22	2081
183-m seine	38.5	B–F	D	1–12	1992,[2] 1993–95	1585	458
61-m block net	3.2	D	D, N, C[2]	1–12	1990,[2] 1991–92	148	210
6.1-m trawl	3.2[3]	B–F	D	1–12	1989,[2] 1990–95	32 (2)	2249
Bay-wide, stratified random station survey							
21.3-m seine (340 m^2)[1]	3.2	A–E,F[2]	D, N, C	3–6, 9–12	1989–95	35	1146
21.3-m seine (70 m^2)[1]	3.2	(A–E),[2] F	D, N, C	3–6, 9–11, 12[2]	1989–95	31	639
21.3-m seine (140 m^2)[1]	3.2	A–F	D, N, C	3–5, 6,[2] 9–12	1989–95	16	1464
6.1-m otter trawl	3.2[3]	A–F	D, N, C	3–6, 9–12	1989–95	3 (5)	2538
184-m gill net	75–150	A–E, F[2]	N, C	3–6, 9–12	1989–93	952	427
198-m gill net	50–150	A–E	N, C	3–5, 9–11	1994–95	583	160
Little Manatee River survey							
9.1-m seine	3.2	—	D	1–12	1989–91	15	77
22.9-m seine	3.2	—	D	1–12	1988–91	115	1460
120-m seine	3.2	—	D	1–12	1990–91	148 (1)	89
Gulf Beach survey							
22.9-m seine	3.2[3]	—	D	1–12	1992–94	156	435
Total						4843 (14)	

[1] Value in parentheses indicates area swept by each haul; see text for further details.

[2] Less than 30 tows for this sampling unit.

[3] Minimum mesh size used.

[4] C = late-metamorphic, juvenile, and older stages; (c) = early- or mid-metamorphic (leptocephalus) larvae.

between January 1988 and December 1991 (Table 1; Fig. 1A). Samples were collected biweekly with a 22.9-m seine at six fixed shoreline stations located between the river mouth and the freshwater zone, with 2–3 seine hauls per station. Supplemental samples were collected with 9.1- and 22.9-m seines at two additional stations from January 1989 to June 1991 and with a 120-m seine at five fixed sites near the river mouth from March 1990 to November 1991.

Gulf beaches—Tampa Bay In a third independent survey by FMRI staff, two beach sites were sampled along the Gulf of Mexico coast of Pinellas County, FL (Table 1; Fig. 1A). Samples were collected biweekly with a 22.9-m seine from September 1992 to November 1994 at Treasure Island and from August 1993 to November 1994 at Indian Shores. Five hauls were made in the surf zone at each site during a single day; each haul began 50 m from the water's edge and proceeded perpendicular to shore.

Lagoon-wide Survey—Indian River Lagoon We also examined data from an FMRI survey of the Indian River Lagoon fishes. The same general sampling design and gear were used in this survey and the bay-wide survey of Tampa Bay (see above; Tables 1–2; Tremain and Adams, 1995). Although the Indian River Lagoon survey started slightly later than the Tampa Bay survey, they were largely contemporaneous (1990–1995). The northern Indian River Lagoon system is a complex of the Indian River basin (zones A–C) and the Banana River basin (zones D–E; Fig. 1B). The sampling program in the Indian River Lagoon differed slightly from that in Tampa Bay. In the Indian River Lagoon, neither the 183-m seine nor block nets were used; gill nets were used at fixed stations and stratified-random locations, and fewer total hauls were made with most types of sampling gear because portions of this program started one or two years later than Tampa Bay's program.

Abundance and size of ladyfish

Monthly relative abundance was calculated as the mean number of ladyfish per haul (including hauls with no ladyfish) for each gear type used in each survey. Data from the stratified random programs were not included in calculations of monthly relative abundance because stratified

Table 2

Sources and details of data examined from the Indian River Lagoon: sampling gear used (with mesh size); geographic zones sampled (see Fig. 1B); diel periods (D=day; N=night; C=crepuscular); months (1=January); years sampled during 1989–95; number of ladyfish collected (C (c)[4]); and total number of sets with each net (f).

Gear	Mesh (mm)	Zones	Period	Months	Years	C (c)[4]	f
Lagoon-wide fixed-station survey							
21.3-m seine (340 m^2)[1]	3.2	A, C–D	D	1–12	1991–95	273 (367)	619
21.3-m seine (70 m^2)[1]	3.2	B,[2] C, E[2]	D	(1–12)[2]	1991–95	403 (67)	235
21.3-m seine (140 m^2)[1]	3.2	A, C–E	D, C[2]	1–12	1991–95	42 (96)	668
6.1-m trawl	3.2[3]	C–E	D	1–3 (4–5),[2] 6–12	1991,[2] 1992–95	7 (1)	617
184-m gill net	75–150	A, D	C	(1–12)[2]	1991–93, 1994[2]	95	202
198-m gill net	50–150	A–D	C, N[2]	(1–12)[2]	1994–95	153	126
Lagoon-wide stratified, random station survey							
21.3-m seine (340 m^2)[1]	3.2	A–E	D, N, C	3–5, 9–11	1990–95	232 (218)	977
21.3-m seine (70 m^2)[1]	3.2	D[2]	D,[2] C[2]	5[2]	1990[2]	0	6
21.3-m seine (140 m^2)[1]	3.2	A–E	D, N, C	3–5, 9–11	1990–95	46 (61)	1286
6.1-m otter trawl	3.2[3]	A–E	D, N, C	3–5, 9–11	1990–95	1 (5)	1501
184-m gill net	75–150	A–E	N, C	3–5, 9–11	1990–93	392	319
198-m gill net	50–150	A–E	N, C	3,[2] 4, 5,[2] 9,[2] 10,[2] 11	1994–95	457	168
Total						2101 (815)	

[1] Value in parentheses indicates area swept by each haul; see text for further details.

[2] Less than 30 tows for this sampling unit.

[3] Minimum mesh size used.

[4] C = late-metamorphic, juvenile, and older stages; (c) = early- or mid-metamorphic (leptocephalus) larvae.

random sampling did not occur in all months of the year. Geographic distributions of age-0 ladyfish were also plotted for each of four seasons. For such maps, relative abundance was calculated as the number of age-0 ladyfish per haul. Values for all positive catches were categorized into four quartile classes (≤25th, 26–50th, 51–75th, and >75th percentile) before plotting, to standardize the data among gear types and estuary. Maximum size criteria were adjusted for each season to exclude fish older than age 0.

Fish size was measured and reported as standard length (SL) in mm. At least 20 randomly selected ladyfish per sample were measured and unmeasured fish were proportionally adjusted for the length-frequency plots to reflect the size structure of the entire sample(s). Sizes from the literature are also reported as SL and were converted when necessary by using the equations $SL = -0.772 + 0.787$ (*total length*), or $SL = -2.46 + 0.943$ (*fork length*); each equation was based on least squares regressions of measurements from 75 ladyfish that ranged from 39 to 475 mm SL (r^2=0.99). Mean salinity at capture was calculated for each 25-mm-SL interval, and for each estuary separately, by using the weighted formula

$$\bar{Y}_w = \left(\sum^{n} w_i Y_i \right) \Big/ \sum^{n} w_i ,$$

where w_i = the number of ladyfish per 25-mm-SL interval for collection i;

Y_i = the salinity measured for collection i; and

n = the total number of collections with fish in that 25-mm-SL interval for that estuary.

We identified early life stages of ladyfish from their size and appearance. The following criteria and general terminology are from Gehringer (1959). Early-metamorphic larvae have a leptocephalus form, with a clear and laterally compressed body. Early-metamorphic larvae shrink as they develop from about 45 to 25 mm SL. Mid-metamorphic larvae are generally less than 25 mm SL. Mid-metamorphic larvae shrink to about 18 mm SL and then grow to about 25 mm SL; they lose the leptocephalus form by the end of this stage. Late-metamorphic larvae have a juvenile form and grow from about 25 mm to 60 mm SL. After 60 mm the age-0 fish are referred to as juveniles. Older age classes (i.e. age-1 or age-2+) are defined by size, as inferred from length-frequency analysis. The complete size and age range of ladyfish in estuaries is not defined in Gehringer or other published literature but appears to be largely restricted to immature fish.

Results

Tampa Bay

In the bay-wide survey, 4422 ladyfish were captured in 7525 21.3-m-seine hauls, 458 183-m-seine hauls, 210

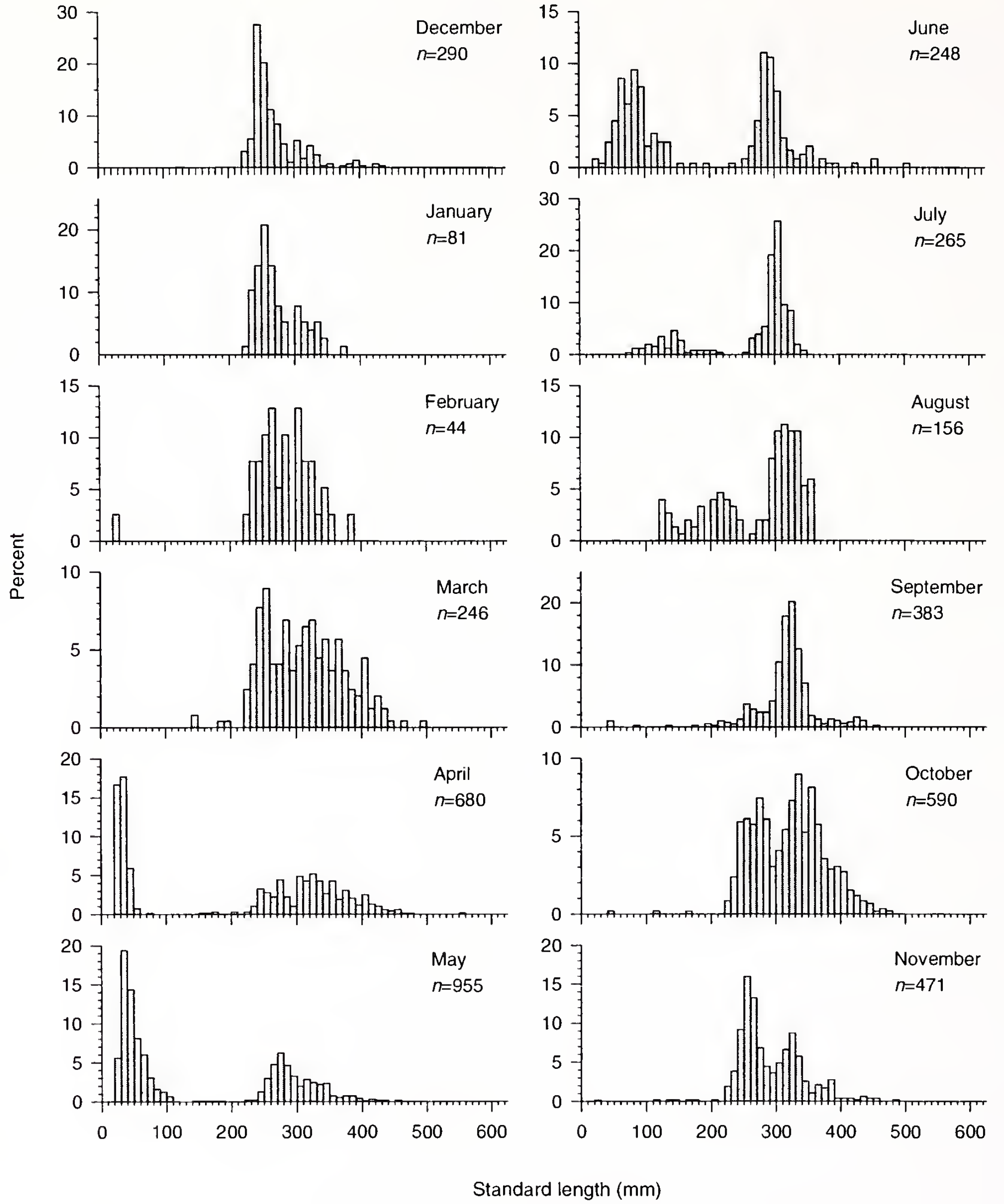

Figure 3

Monthly length-frequency of ladyfish, *Elops saurus*, in Tampa Bay. Early- and mid-metamorphic larvae were excluded because length decreases as they develop. Bay-wide data for all years (1989–95) from all zones (A–F) are plotted. *n* = number of fish sampled.

blocknet sets, 4787 trawl hauls, and 587 gillnet sets (Table 1). Generally, a new cohort of late-metamorphic larvae first appeared in April (Figs. 3 and 4A). Only 13 early- or mid-metamorphic larvae were collected (26–34 mm SL) in the bay-wide survey (n=14 for all Tampa Bay samples). Although the arrival of metamorphosing larvae (largely late-metamorphic stages) was concentrated in April, isolated individuals 26–41 mm SL were collected as early as February (1989) or March (1994, 1995) or as late as September (1994), October (1993), or

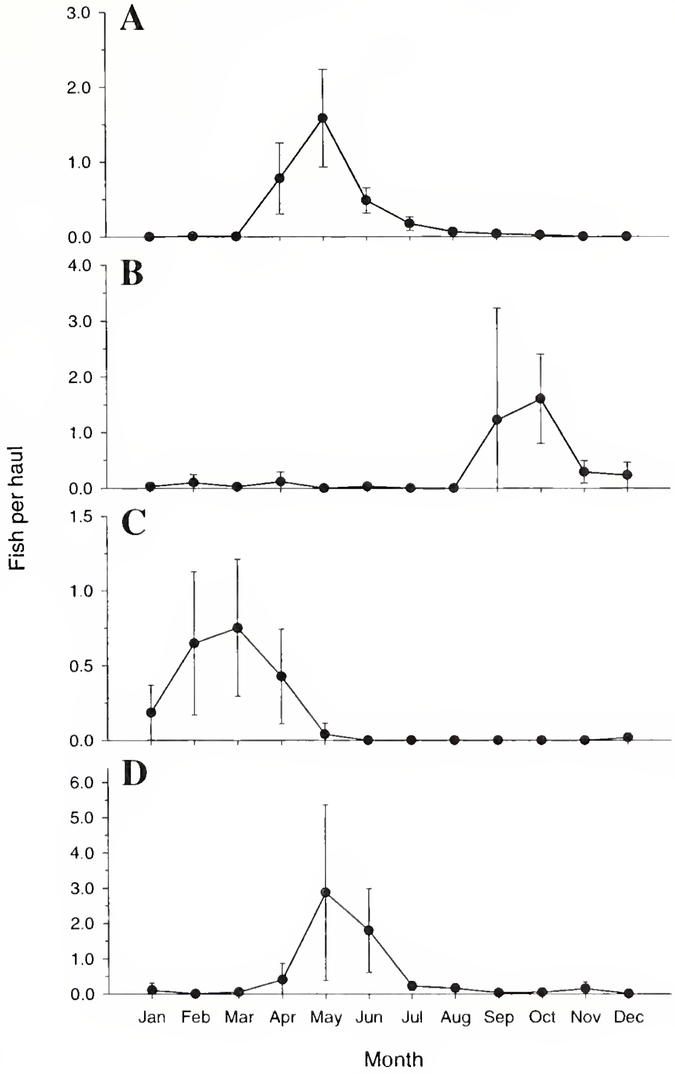

Figure 4

Monthly catch-per-unit-of-effort (mean number per haul ±2 standard error bars) for ladyfish, *Elops saurus*, collected with 21-m and 23-m seines at fixed stations during 1989–1995 in Tampa Bay or 1990–95 in the Indian River Lagoon. (**A**) Little Manatee River (early- and mid-metamorphic larvae are excluded); (**B**) Gulf of Mexico beaches; (**C**) Indian River Lagoon leptocephali (i.e. only early- and mid-metamorphic larvae); (**D**) Indian River Lagoon late-metamorphic and juvenile ladyfish (i.e. early- and mid-metamorphic larvae excluded).

November (1990). All ladyfish ranged in length from 20 to 550 mm SL (Fig. 5). Length-frequency analyses suggested that there were three age classes: age-0 fish overwintering at 200–300 mm SL, age-1 fish overwintering at 300–400 mm SL, and age-2+ at sizes of 400 mm SL or larger (Fig. 3).

Nearly all ladyfish <200 mm SL were collected in mesohaline and oligohaline areas of rivers (zone F; Fig. 6). Early- and mid-metamorphic larvae were collected at polyhaline salinities (mean=20 ppt, Fig. 7); these larvae had probably entered Tampa Bay recently. In Little Manatee River, ladyfish were most common (59.7% of the total individuals) in the mesohaline zone (5.1–18 ppt), less common in the oligohaline zone (31.9%; 0.5–5.0 ppt), uncommon in the polyhaline zone (6.7%; 18.1–30 ppt), and rare in fresh water (1.7%; <0.5 ppt). Abundance in oligohaline waters peaked during June, about one month later than peak abundance in mesohaline waters.

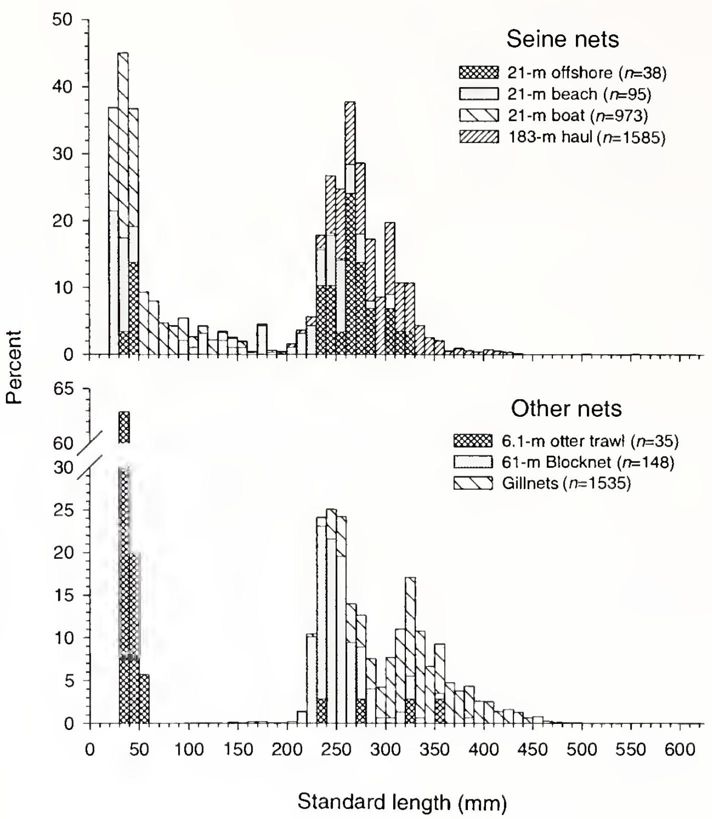

Figure 5

Length-frequency of ladyfish, *Elops saurus*, in Tampa Bay, plotted by sampling gear type. Early- and mid-metamorphic larvae were excluded. Data for all years (1989–95) and all zones (A–F) of bay-wide sampling were plotted. *n* = number of fish sampled.

During autumn, when age-0 fish were <300 mm SL, they began to move out of the rivers (Fig. 8). Fish measuring 170–360 mm SL (5–95 percentile; *n*=55 fish) were found at the mouth of the Little Manatee River during the winter. Large concentrations of age-0 ladyfish were also found at other river mouths, near the dredged (>12 m) portions of the Alafia River, and near the mouth of Tampa Bay. There was a sudden pulse of ladyfish along Gulf of Mexico beaches during September–October (Fig. 4B). These were juvenile age-0 ladyfish measuring 219–260 mm SL (5–95 percentile; *n*=126). Only 2 of 158 individuals collected at Gulf beaches were smaller than 178 mm SL. The absence of larval ladyfish in shallow Gulf beach habitats contrasted strongly with the abundance of larval ladyfish in riverine habitats of Tampa Bay.

Indian River Lagoon

A total of 2916 ladyfish were captured in 3791 21.3-m seine hauls, 2118 trawl hauls, and 815 gillnet sets (Table 2). The seasonal pattern of arrival was similar to that in Tampa Bay. Early- and mid-metamorphic larvae were much better represented (28% or *n*=815) in the Indian River Lagoon than in Tampa Bay (0.2% or *n*=14), and these leptocephali were present from at least December to May (Fig. 4C). Late-metamorphic larvae were found in many months throughout the year, but they were most abundant during spring (Figs. 4D and 9). All ladyfish collected in the Indian River Lagoon ranged from 20 to 600 mm SL. Age-0 fish reached a modal length during winter (i.e. 250–270 mm SL), similar to that observed in Tampa Bay. Age-1 ladyfish appeared to overwinter at a mode of about 350 mm SL, and a small number of age-2+ were probably present as postulated for Tampa Bay.

Ladyfish <100 mm SL were more common in the Indian River basin than in the Banana River basin, and fish >300 mm SL were more common in the Banana River basin than in the Indian River basin (Fig. 10). Juveniles first occupied upper mesohaline and lower polyhaline salinities at sizes of about 75 mm SL, and they remained at such salinities until reaching about 300 mm SL (Fig. 7). In the Indian River Lagoon, the mean salinity occupied by ladyfish 75–125 mm SL was about 5 ppt higher than in Tampa Bay, but mesohaline and oligohaline habitats are less common in

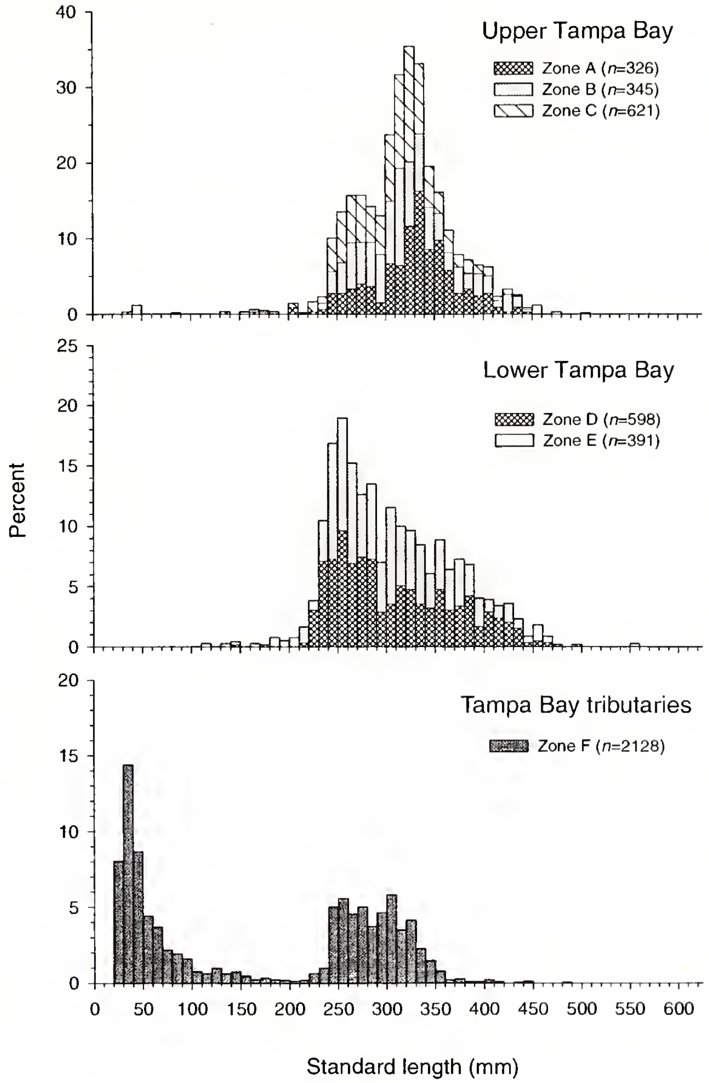

Figure 6

Length frequency of ladyfish, *Elops saurus*, plotted by sampling zones in Tampa Bay. Early- and mid-metamorphic larvae were excluded. Data for all years (1989–95) of bay-wide sampling were plotted. *n* = number of fish sampled.

Indian River Lagoon than in Tampa Bay. Overall, however, ladyfish were broadly distributed in both the Indian River and Banana River basins throughout the year (Fig. 11).

Discussion

Ladyfish were common, widely distributed, and fast growing in both Tampa Bay and the Indian River Lagoon. Metamorphosing larvae and overwintering juveniles were linked together in a single study by using multiple sampling gears. Ladyfish ages inferred from length frequencies indicated that few fish older than 2–3 years were present in either estuary. Over a thousand ladyfish gonads from Tampa Bay and the Indian River Lagoon were examined macroscopically, and nearly all fish were found to be immature (McBride, pers. obs.). Overall, our observations agree with previous reports that ladyfish arrive in coastal embayments as metamorphosing larvae and leave after about 2–3 years to mature and eventually spawn at sea. Carles' (1967) samples from a hypersaline Cuban lagoon contained only immature age 1–3 fish (115–375 mm SL). Others who have suggested that ladyfish mature at sea, where they reach a maximum age of about 6 years and a maximum length of 570–660 mm SL, include Hildebrand (1963), Palko (1984), and Santos-Martinez and Arboleda (1993).

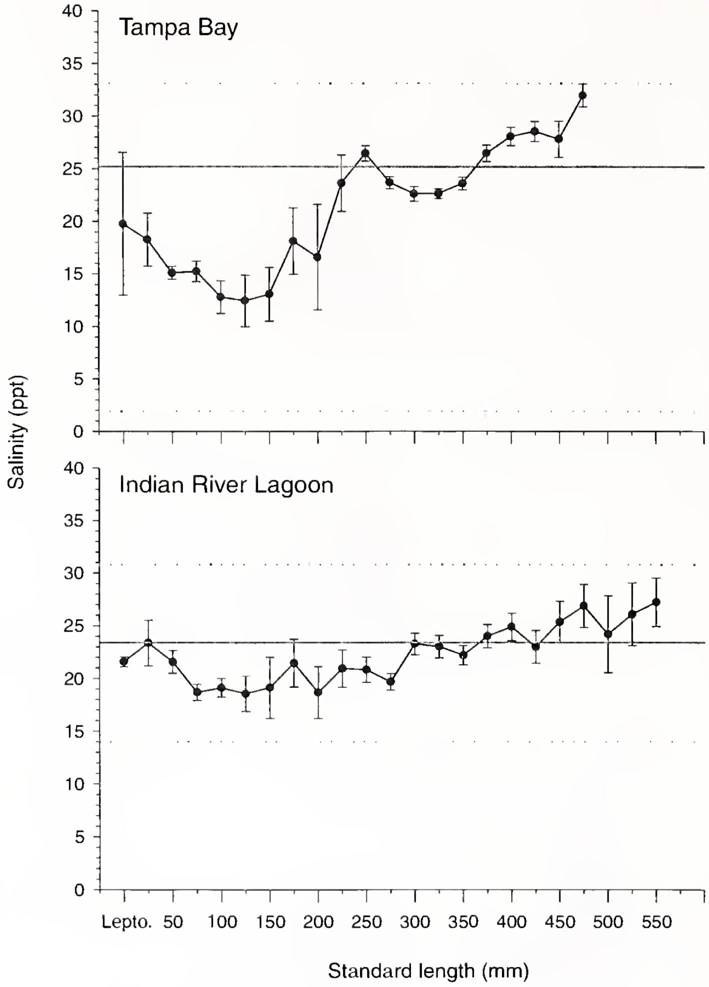

Figure 7

Density-weighted mean salinity at capture for different sizes and stages of ladyfish, *Elops saurus*, in Tampa Bay (1989–95) and Indian River Lagoon (1990–95). Early- and mid-metamorphic larvae are indicated as "Lepto." Error bars represent 2 standard errors. The solid horizontal line represents the median salinity value, and the dotted horizontal lines represent the 10th and 90th salinity percentiles, based on all samples (with or without ladyfish) from each system.

Ladyfish in both Florida estuaries followed a similar sequence of size-specific movements with respect to salinity. Soon after metamorphosis and for much of their first year, ladyfish occupied waters that were lower than the median salinity of each estuary. Following their first winter, ladyfish occupied higher than median salinities. The available salinity range was wider in Tampa Bay and ladyfish were found in a wider range of salinity in Tampa Bay than ladyfish in the Indian River Lagoon. Some details of these movements deserve further investigation. For example, as juveniles grew to about 150 mm SL during summer they were found in progressively lower salinities. However, they may have passively remained in shallow-water habitats where salinity was decreasing because of summer rains, instead of actively selecting lower salinity waters. We also did not examine other factors that may covary with salinity; therefore we cannot rank the effect of salinity in relation to other variables. In one such simultaneous analysis, Friedland et al. (1996) showed that juvenile menhaden (*Brevoortia tyrannus*) select waters of high chlorophyll-*a* levels more consistently than a specific salinity value.

Temperature presumably affects ladyfish distributions as well. During colder than average winters, ladyfish have experienced hypothermal mortality in both Tampa Bay (Springer and Woodburn, 1960) and the Indian River Lagoon (Snelson and Bradley, 1978). We observed ladyfish moving into deeper water, to river mouths, to the lower

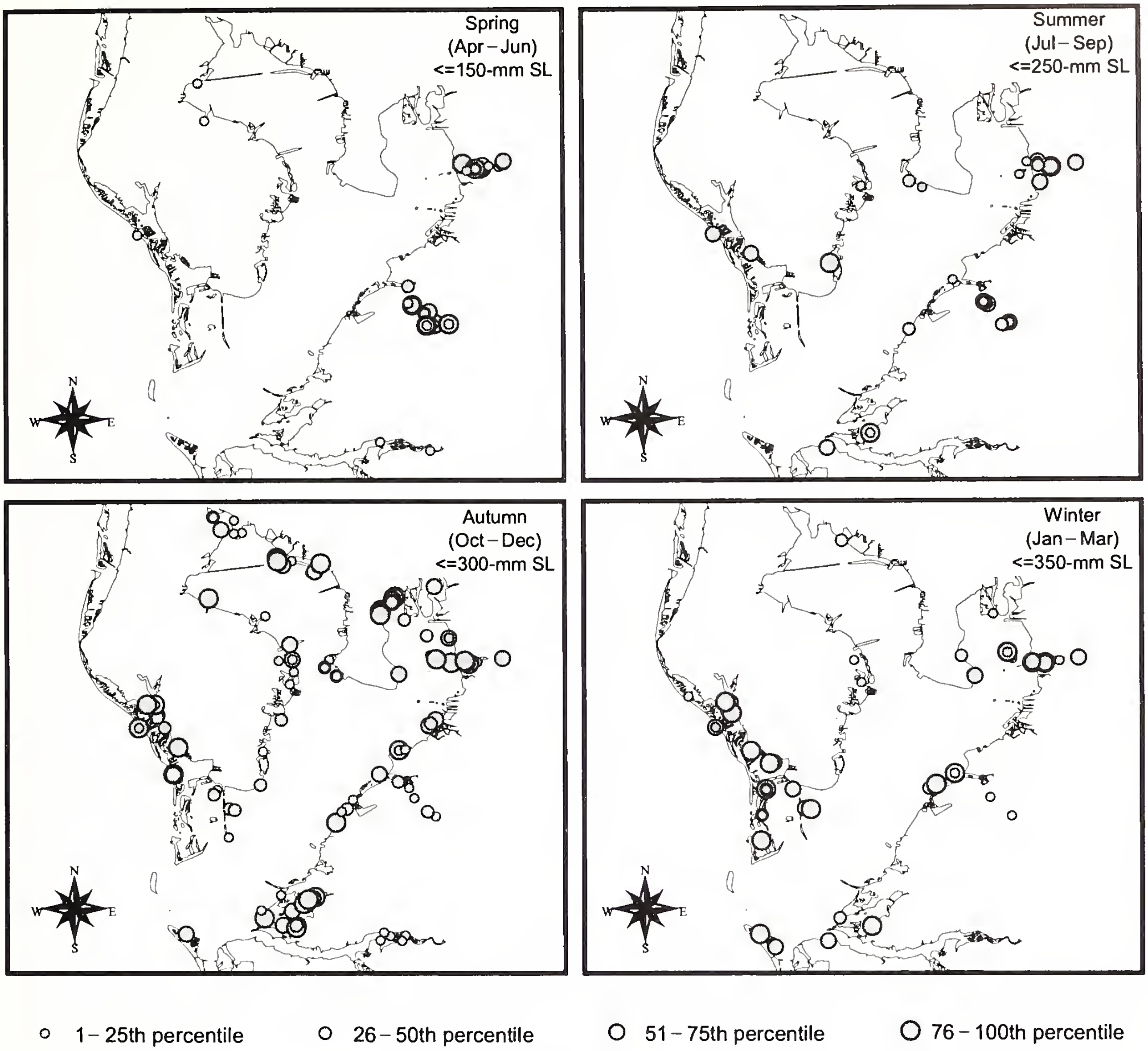

Figure 8

Seasonal distribution plots for age-0 ladyfish, *Elops saurus*, collected in Tampa Bay, 1989–95. Catch-per-unit-of-effort data from all positive catches of age-0 ladyfish were plotted as four quartile classes to standardize the data among gear types; increasing symbol size indicates higher catch per unit of effort. Smaller-size, age-1 ladyfish were excluded by increasing the size range for age-0, as indicated in each plot, from spring to winter.

part of Tampa Bay, and out into Gulf of Mexico waters during colder months. Ladyfish may be selecting these areas for overwintering because such areas are less affected by atmospheric cold fronts. The critical temperatures for ladyfish survival have not been examined in the laboratory, but the above field studies suggest that they could be as low as 10°C. Further work remains on the processes of habitat selection by ladyfish within estuaries.

This description of ladyfish early life history satisfies the first two of three predictions for an estuarine-dependent species: 1) predictable use of estuarine habitats, 2) absence of fish in suitable alternative habitats, and 3) demonstration of a negative population impact caused by the loss of estuarine habitats. Age-0 ladyfish followed an ontogentic migration along a salinity gradient and larval stages of ladyfish were absent from Gulf beaches. The third prediction, that of a negative impact on the population by the absence of habitat, was not supported by findings in our study. We anticipated that salinity differences between Tampa Bay and the Indian River Lagoon might affect growth rates, either due to osmoregulatory stress or perhaps to other correlated factors. Metamorphosing larvae moved into both estuaries at about the same time (i.e. in spring) but age-0 fish attained similar lengths

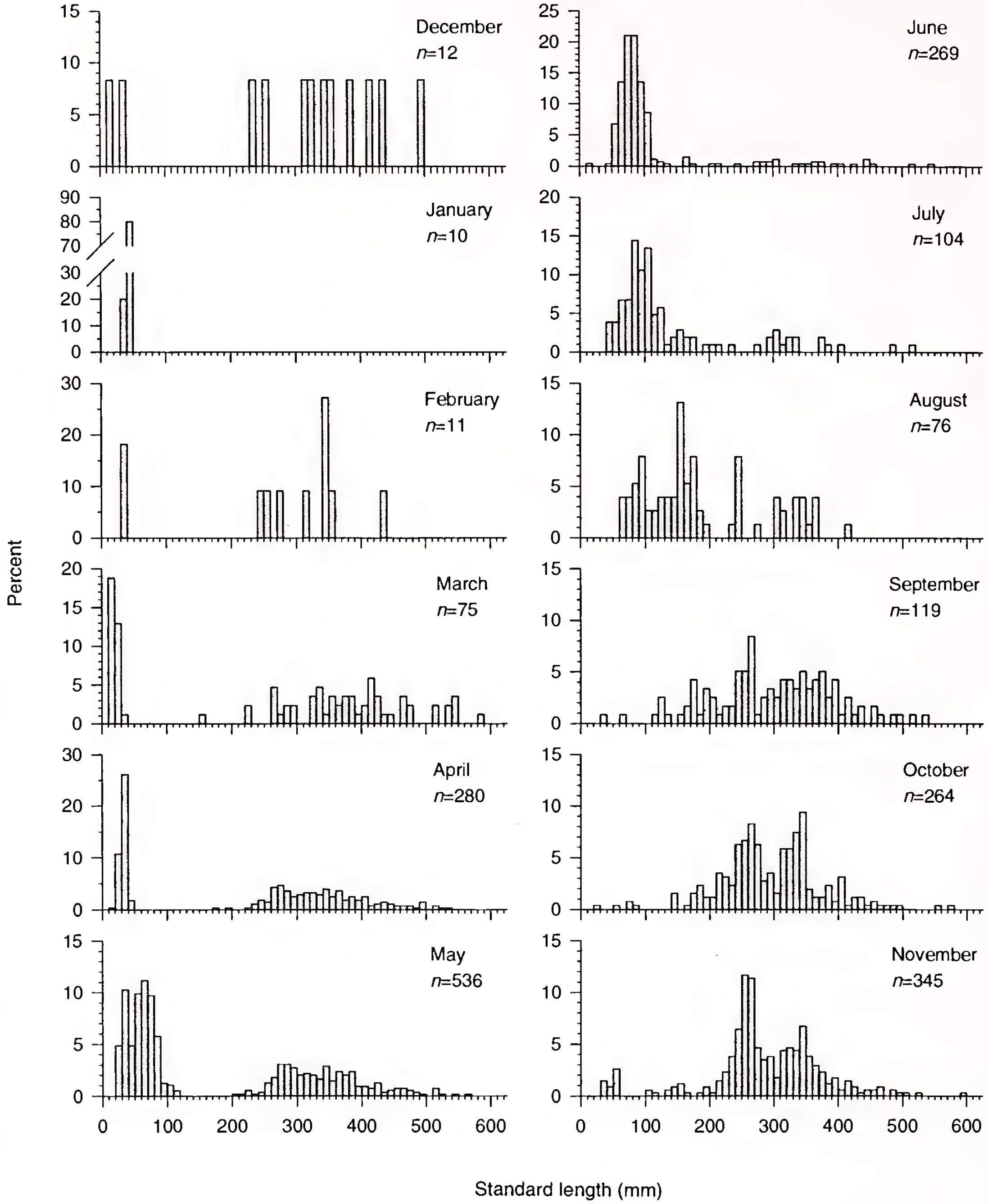

Figure 9

Monthly length frequency of ladyfish, *Elops saurus*, in the Indian River Lagoon. Early- and mid-metamorphic larvae were excluded because length decreases as they develop. Data from all years (1990–95), all gear types, and sampling zones (A–E) were plotted. *n* = number of fish sampled.

(250–300 mm SL) by winter. In Florida Bay, age-0 ladyfish entered estuaries at similar stages during spring and reached similar overwintering sizes as well (Roessler, 1970). In constrast, Carles (1967) estimated much slower growth rates for ladyfish residing in hypersaline lagoons of Cuba, with sizes at annulus formation of 130 mm SL at annulus I, 195 mm SL at annulus II, and 247 mm SL at annulus III. If the growth increments observed by Carles (1967) are indeed annual (he used unvalidated scale annuli), then hypersaline (>35 ppt) conditions may

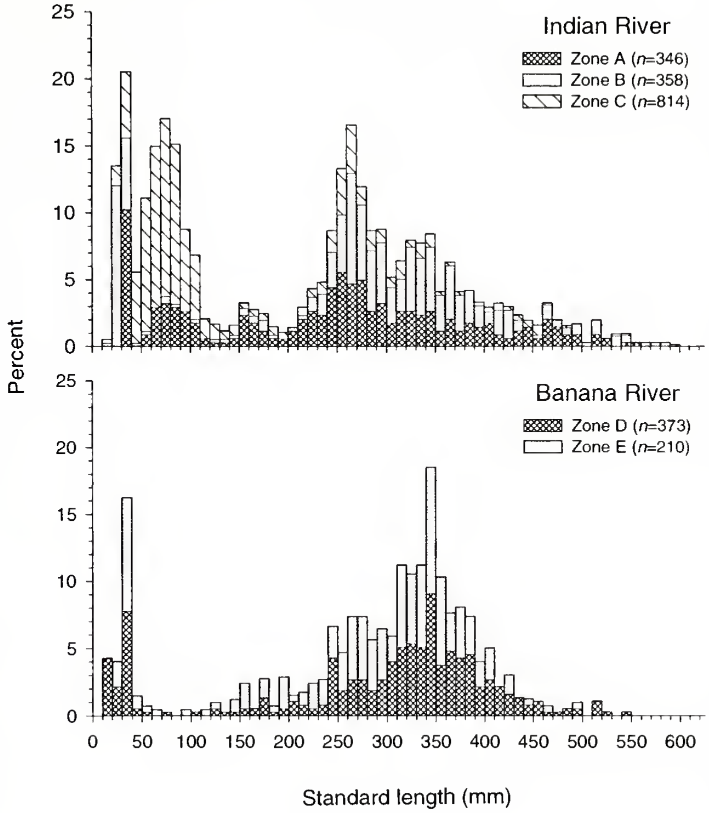

Figure 10

Length frequency of ladyfish, *Elops saurus*, plotted by sampling zones in the Indian River Lagoon. Banana River and Indian River basins are identified in Figure 1B. Early- and mid-metamorphic larvae were excluded. Data from all years (1990–95) and gear types were plotted. *n* = number of fish sampled.

reduce ladyfish growth rates. The timing of ingress into the Cuban estuaries by metamorphosing larvae was not reported by Carles (1967); therefore it is not clear if overwintering sizes are comparable (i.e. if they represent the same seasonal growth period). If ingress into the Cuban estuaries occurred later than spring (i.e. when ladyfish entered Florida estuaries), then this would shorten the length of the growing season and could explain Carles' (1967) results. This preliminary attempt to link salinity to growth rate, although suggestive, requires verification of reduced growth rates in hypersaline conditions. Experimental studies to determine the optimal salinity for ladyfish growth and survival will clarify whether ladyfish benefit by actively selecting low-salinity habitats. Such information would be the last step in demonstrating that ladyfish depend on estuaries and for showing the relative value of positive estuaries (with low salinity areas) to negative estuaries (with hypersaline conditions) for ladyfish populations. It would also be the next step for defining the essential fish habitat of ladyfish and for predicting the effects of changes in estuarine salinity on this fishery species.

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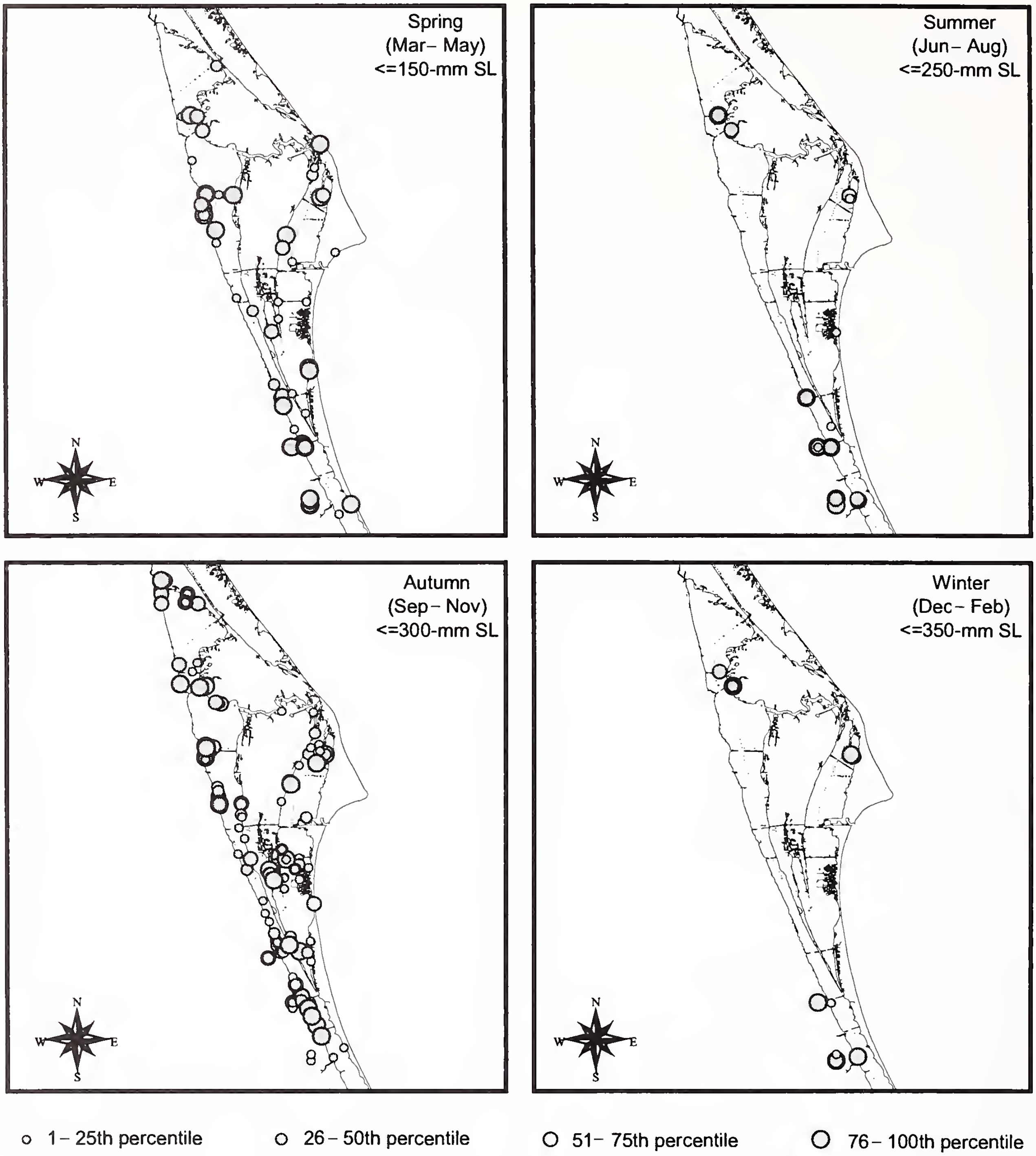
Spring
(Mar– May)
<=150-mm SL
Summer
(Jun– Aug)
<=250-mm SL
Autumn
(Sep– Nov)
<=300-mm SL
Winter
(Dec– Feb)
<=350-mm SL
N
W
E
S
o 1– 25th percentile
o 26– 50th percentile
O 51– 75th percentile
O 76– 100th percentile

Figure 11

Seasonal distribution plots for age-0 ladyfish, *Elops saurus*, collected in the Indian River Lagoon, 1991–95. Banana River and Indian River basins are identified in Figure 1B. Catch-per-unit-of-effort data from all positive catches of age-0 ladyfish were plotted as four quartile classes to standardize the data among gear types; increasing symbol size indicates higher catch per unit of effort. Smaller-size, age-1 ladyfish were excluded by increasing the size range for age-0, as indicated in each plot, from spring to winter.

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The validity of the fin-ray method of age determination for lingcod (*Ophiodon elongatus*)

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Abstract—Oxytetracycline (OTC) injections were used in a mark-recapture experiment undertaken to validate the fin-ray method of age determination of lingcod (*Ophiodon elongatus*). In most cases, the number of annuli that formed beyond the OTC mark corresponded to the number of years at liberty. Expected and interpreted annuli counts from fin rays matched in 94%, 92%, 91%, and 75% of lingcod at liberty for one, two, three, and four years, respectively. These results validate the annual pattern of banding in fin-ray sections of fish up to 18 years of age. One of the consequences of not establishing and following age determination criteria is illustrated by the change in age composition due to misidentification of the first few annuli. A change in age composition has implications for stock estimates, such as mortality rates, and harvest strategies. For example, overfishing could result from underestimated mortality rates if fishery managers used a strategy that set fishing mortality equal to natural mortality. It is recommended that age determination criteria be routinely assessed to provide reliable age estimates.

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Lingcod (*Ophiodon elongatus*) are distributed off the west coast of North America in the nearshore waters from Baja, California, to the Shumigan Islands, Alaska. They have been an important component of the commercial fishery off British Columbia since the mid-1880s and a favorite target of recreational anglers since the 1950s. They inhabit nearshore waters and are commonly found along the bottom at depths ranging from 3 to 400 m; however most are found in rocky areas 10 to 100 m. Growth during the first years of life is rapid and up to age 2 it is similar for males and females, both reaching an average length of 45 cm. After age 2, females grow faster than males and the growth of males tapers off at about age 8, whereas females continue to grow rapidly until about age 12–14. For waters off the west coast of Canada, the maximum age recorded for lingcod has been 14 years for males and 20 years for females. Females reach lengths in excess of 100 cm, whereas males rarely exceed lengths of 90 cm.

The fin-ray method for age determination has been used for a wide range of freshwater and marine species such as sturgeon (*Acipenser* spp.), Pacific salmon (*Oncorhynchus* spp.), brown trout (*Salmo trutta*), whiter sucker (*Catostomus commersoni*), lake whitefish (*Coregonus clupeaformis*), channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), ide (*Leuciscus idus*), tuna (*Thunnus* spp.), rudd (*Scardinius erythropthalmus*), chub (*Squalis cephalus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), and yellow perch (*Perca fluviatilus*) (Beamish 1981). However, in only a few instances have there been attempts in recent years to validate age estimates with the fin-ray method (Rien and Breamesderfer, 1994; Rossiter et al., 1995; Stevenson and Secour, 2000) despite the caution posed by Beamish and McFarlane (1983) that validation of age determination techniques is difficult, albeit critical.

Beamish and Chilton (1977) developed a method of aging lingcod that used thin sections of fin rays combined with mean annular diameter measurements to locate the position of the first and second annuli. This technique allowed estimates of mortality rates, growth rates, and fecundity to be determined for each age group. These rates strongly influenced assessment and management approaches for lingcod. Preliminary validation of the method was accomplished by using a mark-recapture experiment in conjunction with injections of oxytetracycline (OTC) (Cass and Beamish, 1983). However, this preliminary validation of the method was based on only four recoveries. A second experiment was initiated in 1982 to complete the validation of the fin-ray method for lingcod. Our report presents the results of this second mark-recapture study combined with OTC injections.

During the course of analyzing lingcod age data in the early 1990s, it became apparent that the annular diameter measurement criteria suggested by Beamish and Chilton (1977) were no longer being followed. Resulting parameter estimates based on these age estimates had changed. This resulted in a misunderstanding of growth, mortality, and age at maturity, which had implications for development of management strategies for lingcod. We therefore also report on a re-examination of the measurement criteria proposed by Beamish and Chilton (1977) and show that accurate ages for lingcod can be produced by using the fin-ray method combined with mean annular diameter measurements.

Methods

During 14–27 July 1982, a major tag and release program was conducted on the commercial fishing grounds, La Perouse Bank (48′45°N; 125′55°W), off southwest Vancouver Island. The primary focus of this tagging program was for validation of age determination. Methods and survey design are outlined in Cass et al. (1983). Prior to processing, all fish were anesthetized with tricaine methane sulfonate (MS222). We measured their fork length (mm) and inserted an individually numbered Floy FD68 anchor tag into the connective tissue just below the anterior base of the first dorsal fin.

We injected the tagged fish with a 25 mg/kg body-weight dosage of oxytetracycline into the interperitoneal cavity (McFarlane and Beamish, 1987). Of the injected fish, a random subsample was kept in 3000-L holding tanks for 48 hours to assess short-term mortality due to the OTC injection, then released. We released a random subsample of tagged fish without OTC injections as a control group to assess long-term (6-month) mortality due to OTC injection by comparing recapture rates for the control fish with those for the OTC-injected fish.

Tagged lingcod were recovered by the commercial fishery off the southwest coast of Vancouver Island. Because the first annuli after the OTC mark would form during the winter of 1982–83, only tagged lingcod recovered after 1 January 1983 were used in the validation of age determination. Returned whole fish were measured for fork length (mm), and a portion of the second dorsal fin was removed for age determination according to Beamish and Chilton (1977). Sectioned fin rays were illuminated with ultraviolet light to fluoresce the OTC mark and with regular light to identify annuli.

All recovered lingcod were aged in June and August of 1987. In October 1987, a change in personnel led to an unknowing change in the criteria for determining the first few annuli. Chilton and Beamish (1982) suggested that known average diameter of the first and second annuli could be used to estimate their location. The first and second annuli can be difficult to determine when resorption of the inner portion occurs or if the center becomes obscured by irregular-shaped deposits of opaque material. In addition, the presence of checks can also make it difficult to identify the first two annuli. In all cases, an estimate of the position of the third annulus can be made by measuring the diameter of the first and second annuli from juvenile fish or fish where these annuli are visible (Chilton and Beamish, 1982). By 1988, the primary reader was no longer using the Chilton and Beamish (1982) criteria. The recovered lingcod were re-aged after this change in criteria and second ages (using these criteria) were compared with original ages (not using these criteria) to assess bias. We compared age compositions of a subsample of the lingcod with the two age estimates to illustrate the consequences of not using the Chilton and Beamish (1982) criteria.

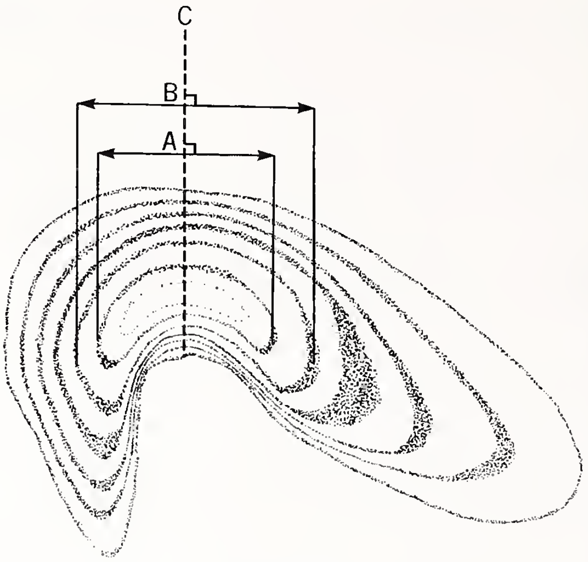

Figure 1

Schematic representation of section of fin ray for measurements of first (**A**) and second (**B**) annular diameters. Measurements should be made along axes perpendicular to the axis (**C**) of the inner groove of the fin ray.

Juvenile lingcod (less than 48 cm) were captured in 1987 in the recreational fishery in the Strait of Georgia. Fork lengths were measured (nearest mm) and dorsal fin rays were sampled during the creel survey program. These juveniles would not likely have resorbed fin-ray centers. The fin rays were sectioned (Beamish and Chilton, 1977), and illuminated with regular light. The diameter to the first and second annuli were measured with a micrometer eyepiece under 40× magnification to the nearest micrometer (μm). Diameter measurements were made along an axis perpendicular to the inner groove of the fin ray (Fig. 1). If an annuli appeared as a thick zone, the range from the beginning to the end of the zone was measured and the median used as the diameter measurement.

Results

A total of 7429 lingcod were tagged and released during July 1982. Of these, 6946 were injected with OTC and 483 were not injected but were released as the control group for assessing long-term (6-month) mortality due to OTC injection. A total of 188 lingcod were held for 48 hours after being tagged and injected with OTC. No mortality occurred prior to release. Within the first six months (i.e. by 31 December 1982) 1442 lingcod were recovered. During this period, the return rates of OTC and non-OTC–injected lingcod were similar (19.5% and 18.4%, respectively) indicating no long-term mortality from OTC injection.

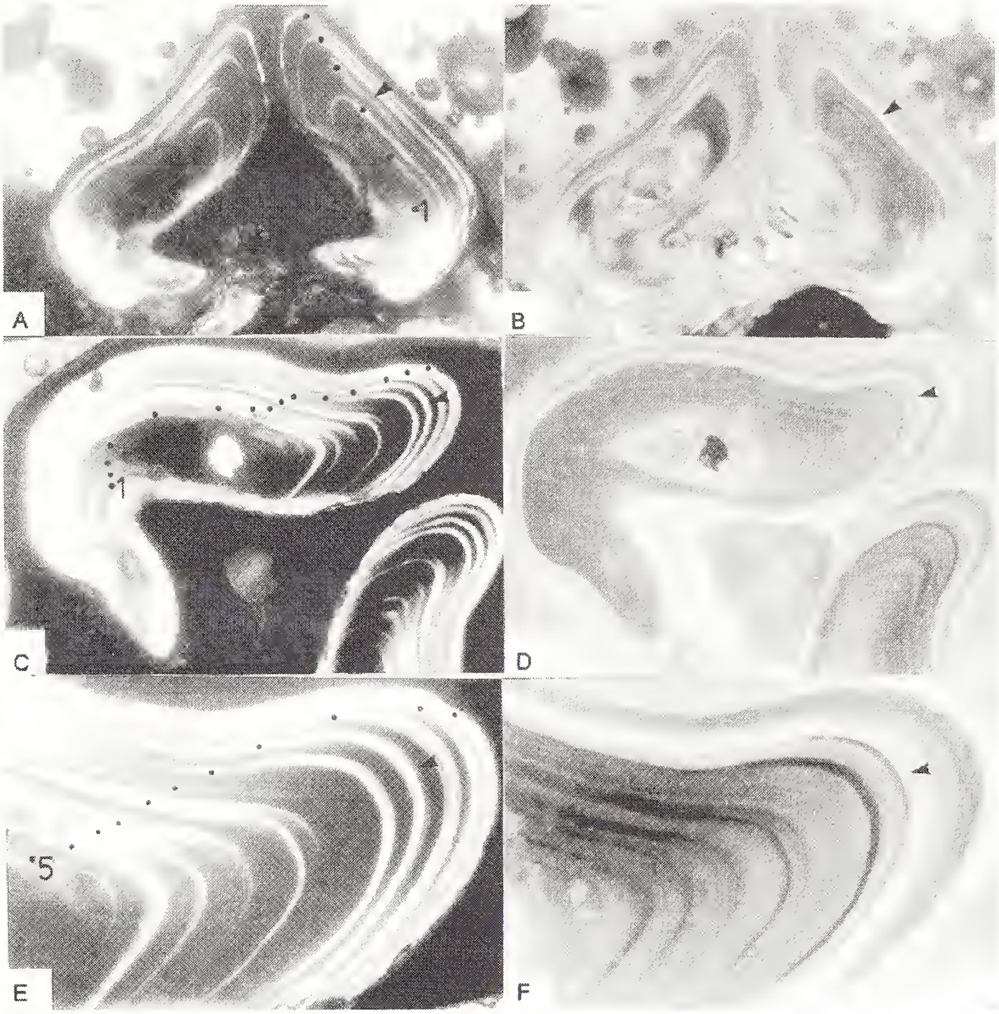

Figure 2

Annuli are indicated by dots, OTC (oxytetracycline) mark indicated by arrow. A, C, E were photographed by using white light and B, D, F were photographed by using ultraviolet light. (**A** and **B**) Fish numbered B8223905 at liberty for 2 years, with OTC mark evident after fourth annulus. Total age was 6+. (**C** and **D**) Fish numbered B8227766 at liberty for 3 years, with OTC mark evident after twelfth annulus. Total age was 15+. (**E** and **F**) Enlargements of sections C and D after the fifth annulus.

Between 1983 and 1986, 1002 tagged lingcod were recovered. From these, 460 lingcod fin rays were obtained for age determination validation: 348 recovered in 1983, 65 recovered in 1984, 43 recovered in 1985, and 4 recovered in 1986. The number of observed annuli after the OTC marks corresponded well to the number of expected annuli based on the number of years at liberty (Table 1). Lingcod recovered in 1983 were expected to have one annulus after the OTC mark and approximately 94% of the samples were estimated to have one annulus. Lingcod recovered in 1984, 1985, and 1986 had estimated annuli after the OTC mark that corresponded to the expected number of annuli in 92%, 91%, and 75% of the samples, respectively.

In most cases, the presence of the OTC mark was easily discernible and appeared as a crisp distinct line. Annuli following the mark were also clearly visible. Fish numbered B8223905 was recovered in July 1984 and aged at 6 years. The OTC mark formed just beyond the fourth annulus (Fig. 2A) and was obvious under ultraviolet light (Fig. 2B). After the OTC mark, two complete annual growth zones were present corresponding to the two years at liberty. Each annual growth zone consisted of an opaque (summer) and a translucent (winter) zone. The OTC mark at the beginning of the summer opaque zone (Fig. 2A) indicated that growth had just begun prior to tagging and injection in July.

Fish numbered B8227766 was recovered in July 1985 and aged at 15 years. As with fish B8223905, the OTC mark was also formed at the beginning of an opaque zone but was a broad band covering the whole summer zone

Table 1

The number of estimated annuli after the OTC mark versus the expected annuli based on years at large. Numbers in bold indicate those samples in which the number of observed annuli equaled the number of expected annuli.

	Expected annuli			
Estimated annuli	1	2	3	4
0	3	1	—	—
1	**328**	3	—	—
2	16	**60**	—	—
3	1	1	**39**	1
4	—	—	4	**3**

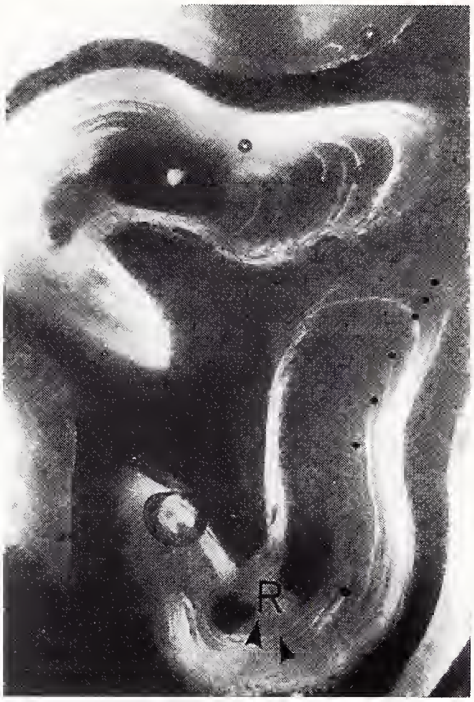

Figure 3

Fish numbered B8224635 had resorbed (R) central portion of the fin ray, making identification of the first two annuli (arrows) difficult. Subsequent annuli are indicated by dots. Total age was 9+.

(Fig. 2C) and clearly visible with ultraviolet light (Fig. 2D). This fin ray had three annual growth zones after the OTC mark, each comprising opaque and translucent zones (Fig. 2C). An annual growth zone can contain several distinct translucent zones, i.e. checks. In this example, the tenth labelled annulus did not appear to be continuous and could, therefore, be interpreted as a check (Fig. 1, E and F). Although this area likely contained checks, the thickness and prominence of the labelled translucent zone around most of the fin-ray section (Fig. 2C) indicated that it was the edge of the tenth annulus.

Fish numbered B8224635 was recovered in July 1985 and was estimated to be 9 years old (Fig. 3). Unlike the previous two examples, the first two annuli were difficult to determine because of resorption in the central portion of the fin ray (Fig. 3). We used the diameter measurement criteria of Chilton and Beamish (1982) to locate the first and second annuli.

The 46 juvenile lingcod available from the creel survey in 1987 were used for first and second annular measurements. The mean first annular diameter was 0.40 mm (SD=0.07) and the mean second annular diameter was 0.63 mm (SD=0.11). With one exception, there was no overlap between the measured diameter for the first annulus and those measured for the second annulus and the two means were significantly different (t-test, t=17.46, df=86, P<0.0001). The mean annular diameter did not vary across the range of fork lengths observed (Fig. 4).

Ages determined by using average diameter for the location of first and second annuli measured in the late-1980s were generally one year older than ages determined without using a measurement to locate the first two annuli (Fig. 5). There appeared to be no trend related to age. However, across all ages, the unmeasured estimates ranged from three years younger to three years older than estimates made with the measurement criteria (Fig. 5). This result is likely due to the exclusion of annuli that cannot be recognized or due to the inclusion of checks, mistaken as annuli, in some fish. There was no difference in the number of annuli estimated after the OTC mark. The one-year aging bias is illustrated in the age composition for the lingcod recovered in 1983 (n=341). The age composition for the ages determined with the measurement criteria had a mode of 6 years, whereas the age composition for ages determined without the measurement criteria had a mode of 5 years (Fig. 6). For all of the samples, the estimated ages ranged from three to 18 years.

Discussion

Accurate age determination is essential for proper stock assessment and fisheries management. Although aging inaccuracies of one year might not be critical in long-lived species (e.g. 80-year-old rockfish), they can have serious stock assessment implications for shorter-lived species, such as lingcod. This is likely true because shorter-lived species typically have only four or five year classes that dominant the fishery. It is therefore important to develop accurate methods for age determination and to validate those methods. The fin-ray method first described by Beamish and Chilton (1977) is an accurate method for age determination of lingcod. Annular growth is represented by an opaque (summer growth) and a translucent (winter growth) zone and the edge of the annulus is assigned to the translucent zone (Beamish and Chilton, 1977). Using

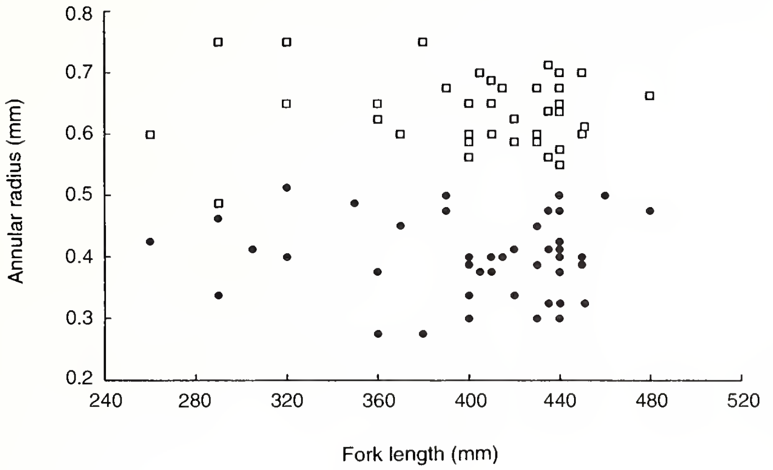

Figure 4

The mean diameter of the first (circles) and the second (squares) annuli are 0.40 mm and 0.62 mm, respectively. The mean diameters do not show a change in value across the fork lengths observed.

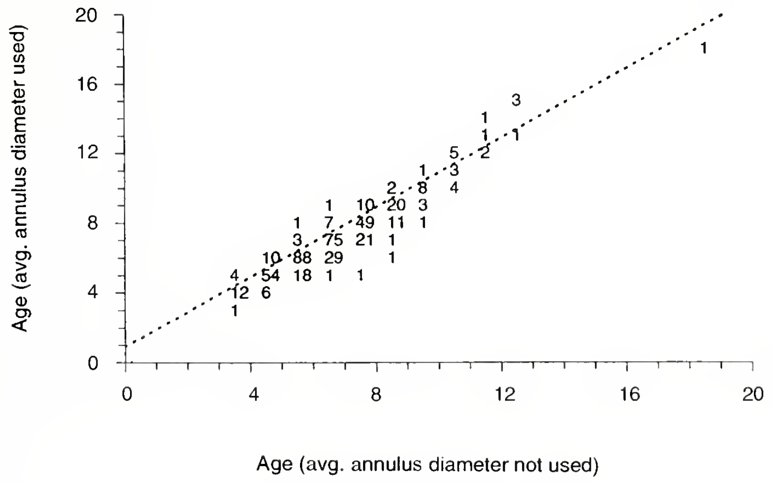

Figure 5

For all recovered lingcod, the ages determined when average annulus diameter measurements were used to estimate the location of the first and second annuli were approximately 1 year greater than ages determined when average annulus diameter measurements were not used (i.e. intercept on regression line=1). Because many data points overlapped, numbers indicate the number of observations at each point.

this method in our validation study, we found that in most cases the number of annuli estimated after an OTC mark corresponded to the number of expected annuli based on years at liberty.

Lingcod, and other species, resorb the central portion of their fin rays, making the identification of the first few annuli difficult in older fish (Beamish and Chilton, 1977). In order to obtain the most accurate ages, mean annular diameter measurements should be used routinely to locate the first and second annuli. We found no significant overlap between the first and second annular diameter measured in juvenile lingcod. It is therefore a useful criterion for age determination and can be used for lingcod inhabiting waters off the coast of British Columbia. The fin-ray method for age determination is routinely applied to other species (Beamish, 1981) and we suggest investigators examine similar criteria to ensure the identification of the first few annuli.

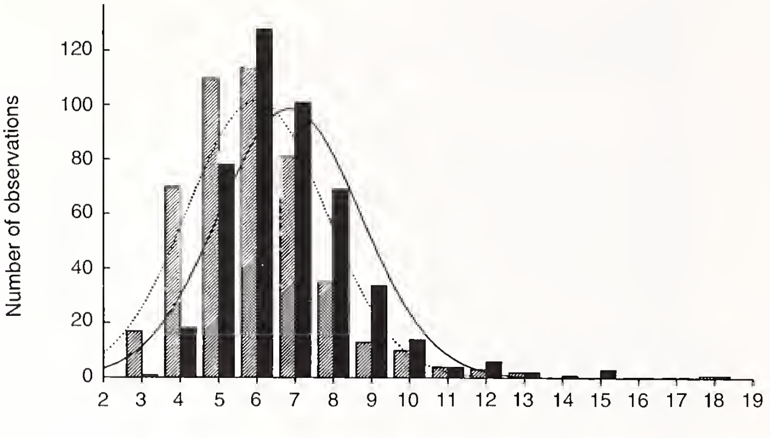

Figure 6

Age composition for the 341 lingcod recovered in 1983 when no measurements were used to estimate location of first and second annuli (striped bars) and when average measurements were used to estimate their location (solid bars).

Consistent use of mean annular diameter for age determination of lingcod will enable the best estimation of ages. We have illustrated that when the established criteria are not applied to identify the first few annuli, systematic underaging of approximately one year occurs—a serious consequence for stock assessment. For example, underestimates of age would overestimate mortality rates. If managers set fishing mortality equal to natural mortality (i.e. $F=M$) to estimate fishery yields, then overfishing would result (Tyler et al., 1989). If recruitment were estimated from a catch-at-age analysis, erroneous age composition and overestimates of mortality would lead to gross overestimates of recruitment (Tyler et al., 1989).

Recently, rapid and major shifts in marine ecosystems have been shown to have consequences for the biology and behavior of a large number of fish species (Beamish et al., 2000). These shifts can occur within a year; therefore it might be expected that growth of some species could change rapidly because of environmental effects. Examination of growth time series would likely indicate a change coincident with environmental changes. However, we have illustrated that a rapid change in a growth time series can also be the result of the change in aging criteria. Therefore, for age determination to be valid and effective in fishery and ecosystem research, the criteria used should be routinely reviewed and verified.

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The behavior, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*) larvae in relation to prey availability: adaptations to an extended larval period

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Abstract—Foraging behavior and prey abundance are significant factors determining the survival success of fish during the larval stage. Witch flounder (*Glyptocephalus cynoglossus*) are reported to have the longest pelagic stage of any northwest Atlantic flatfish. We used laboratory experiments to investigate the behavior and performance of witch larvae in relation to prey availability during this important life history stage. In one experiment, larvae were reared at a range of prey densities (2000, 4000, and 8000 prey per liter) and their growth and survival were monitored for 12 weeks after hatching. In a second experiment the foraging behavior of larvae was recorded during feeding trials at a range of prey densities (250, 500, 1000, 2000, 4000, 8000, and 16,000 prey per liter) during weeks 2–8 after hatching. The larval search strategy for prey appeared to change from one that was saltatory to one that was cruising, and the foraging behavior was not strongly affected by variation in prey availability. The growth rate was rapid (0.53 mm/d) and was unaffected by changes in prey density as was survival. Witch flounder larvae likely have low prey requirements compared with yellowtail flounder and Atlantic cod reared under similar laboratory conditions. The ability to forage effectively when prey is abundant or scarce and the low prey requirements of this species may be an adaptive response to the extended larval period.

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The effects of prey abundance on the behavior, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*, Linnaeus) larvae were examined by using laboratory experiments. Witch flounder is an interesting study organism because it displays a life history characteristic that is very different from other Pleuronectiformes. Most flatfish undergo metamorphosis at a small size and early age (see Miller et al., 1991; Osse and Van den Boogaart, 1997). Little information is available on witch flounder larval biology and ecology, and on precise length and age data at metamorphosis for witch flounder are lacking in the literature. However, field evidence indicates that witch flounder has the longest pelagic stage of any Northwest Atlantic flatfish and undergoes metamorphosis at a relatively large size (Scott and Scott, 1988). Because of their long larval period, witch flounder larvae will likely be exposed to temporal variations in prey abundance at sea. Larvae are likely able to cope with large fluctuations in prey availability and may not be as susceptible to mismatches in prey abundance or starvation in relation to other species. Unfortunately, no information is available concerning the performance of witch flounder larvae in relation to the availability of prey in the wild. Therefore, we used laboratory experiments to gain an understanding of the early biology of this species. The objective of our study was to determine the growth, survival and foraging response of witch flounder larvae to differences in prey abundance.

Materials and methods

Eggs and milt of adult witch flounder in spawning condition were collected aboard a commercial fishing vessel in the Gulf of Maine. The eggs from approximately 10 females were fertilized with milt from an equal number of males. Fertilized eggs were shipped on ice by courier to the Ocean Sciences Centre of Memorial University of Newfoundland in Logy Bay, Newfoundland. Upon arrival, the eggs were stocked in 250-L cylindroconical upwelling incubators at 12°C. Larvae hatched over a one-day period, seven days after fertilization (day 0 of the experiment). At this point, 10 larvae were sampled for morphometric measurements (defined below).

Two experiments were designed to obtain the most information possible from the limited number of larvae available. In experiment 1, larvae were reared at a range of prey densities and their growth and survival were monitored. In experiment 2, larvae were exposed to a range of prey densities and their behavior was recorded.

We recognize that designing ecologically relevant laboratory experiments is difficult (Suthers, 2000). Our rearing conditions were chosen on the basis of established techniques for other north Atlantic marine fish larvae that have resulted in high growth and survival (Puvanendran and Brown, 1999; Rabe and Brown, 2000). Although these rearing conditions do not precisely mimic a natural environment, they do enable valid comparisons of experimental results with those for other species. One can then use this comparative evidence to make speculations concerning observations from the field. We used cultured zooplankton as live prey for the larvae. Although this is not the natural prey for larval witch flounder, it offers desirable characteristics necessary for experimentation, such as uniform shape, size, and swimming speed and although prey densities used in our experiments were typically higher than averages reported from the field (Myers et al., 1994), these levels were necessary in the laboratory to promote growth and survival (see Houde, 1978; Puvanendran and Brown, 1999).

Experiment 1: Growth and survival

Six 33-L rectangular black tanks were used for the rearing experiment. All tanks were kept in a water bath to minimize temperature fluctuations and were supplied with filtered (25 µm) seawater. Each tank was fitted with two air stones that provided light aeration to promote a homogeneous distribution of prey. The light level at the water surface was 200 lux and continuous lighting (24 h) was used. The temperature ranged from 10° to 15°C (mean 12.6°C).

On day zero, newly hatched larvae were transferred to each experimental tank to achieve a stocking density of six larvae per liter. Three replicated feeding treatments were chosen: 2000, 4000, and 8000 prey per liter. Previous results in our laboratory for other northwest Atlantic species suggested that this range of prey densities was sufficient to observe the effects of prey density on larval growth and survival (Puvanendran and Brown, 1999; Laurel et al., in press).

Rotifers (*Brachionus plicatilis*) enriched with culture selco (INVE, Belgium) or *Artemia franciscana* nauplii enriched with DHA selco (INVE, Belgium) or Algamac (Bio-Marine, Hawthorne, CA), were used as prey for the larvae. Prey densities were determined by sampling 5-mL aliquots from different depths within the tanks (below surface, mid-depth, and above bottom) and were adjusted as needed to maintain nominal densities three times a day (around 10 AM, 4 PM, and 10 PM). Microalgae (*Isochrysis* and *Nannochloropsis*) were added to the experimental tanks prior to each feeding.

At week 5, the larvae were transferred to larger 65-L circular tanks because they had grown too large for the smaller tanks; all other protocols remained unchanged. The rearing experiment was stopped at week 12. At this point most larvae had begun eye migration but were still pelagic.

Data collection Larvae were sampled weekly for standard length (SL, measured from tip of snout to posterior end of notochord). For weeks 0–3, larvae were measured to the nearest 0.1 mm with a dissecting microscope. Standard length was measured to the nearest 1 mm after week 3. On weeks 1, 5, 8, and 12, five larvae were lethally sampled (killed by an overdose of MS-222) from each tank for determination of SL, body height (BH, myotome height posterior to anus), and dry weight (DW). These sampled larvae were kept in beakers on ice and measured immediately after death to prevent shrinkage from dehydration. Larvae were rinsed in 3% ammonium formate, placed on preweighed aluminum foils (weighed to nearest 0.001 mg), dried at 55°C for at least 48 hours, and reweighed. For all other weeks, the SL of ten live larvae per tank was measured.

The absolute growth rate was calculated according to the equation

$$G = (L_t - L_0)/t,$$

and the length-specific growth rate (SGR) was calculated according to the equation

$$SGR = (\ln(L_t) - \ln(L_0)/t) \times 100,$$

where L_t = the mean final length (mm);
L_0 = the mean initial length; and
t = the period of growth (days; Busacker et al., 1990).

All tanks were examined for mortalities twice daily from day 14. Dead larvae decomposed too quickly to be observed prior to this time. At the end of the experiment, the number of surviving larvae in each tank was recorded.

Data analysis For each growth measurement (SL, BH, DW), a mean value was calculated for each replicate and this value was used in the analysis. Growth measurements were analyzed by treatment using a model I analysis of covariance (ANCOVA, Zar, 1999) with age of the larvae (weeks after hatching) as the covariate (α=0.05). Dry weight data was logarithmically transformed to satisfy the assumptions of the ANCOVA. A one-way analysis of variance (ANOVA) was used to test for differences in survival at the end of the experiment.

Experiment 2: Behavior

Larvae were stocked into a 250-L cylindroconical upwelling tank on day zero. This tank served as a general rearing tank for larvae used for behavioral observations. The light was continuous (24 h) at an intensity of 200 lux at the surface. Preliminary results with witch flounder larvae showed that this light intensity and light regime resulted in good growth and survival (Rabe, 1999). The temperature ranged from 4–14°C (mean 12.4°C) during the 8-week experiment.

Feeding began on day 1 after hatching. Rotifers or *Artemia* nauplii, or both, were used as prey for the larvae and were enriched with commercial products as described previously. Larvae were fed three times daily at 4000 prey per liter (prey/L). The prey density in the rearing tank ranged from 0–4,000 prey/L throughout the day. Microalgae (*Isochrysis* and *Nannochloropsis*) were also added twice daily.

Data collection Behavioral observations were conducted every 3–4 days from week 2 to 8 beginning on day 8. The prey densities used in the feeding trials were 250, 500, 1000, 2000, 4000, 8000, and 16,000 prey/L. Prior to the

Table 1

Definition of the modal action patterns (MAPs) observed in developing witch flounder larvae, after Barlow (1968).

MAP	Definition
Locomotory MAPs	
Swim:	Forward movement of the larva through the water column resulting from undulations of the caudal region.
Turn:	A rapid lateral bend initiated by the head results in rotating the body approximately 180°.
Nondirected MAPs	
Pause:	Larva is motionless (similar to "non-swimming" of Munk, 1995).
Sink:	Larva is motionless and descends through the water column, often head first.
Shake:	Rapid lateral undulations of the entire larval body.
Foraging MAPs	
Orient:	The head movement towards a prey item (similar to "orientation" of Brown and Colgan, 1985).
Fixate:	The larva is stationary and bends its caudal region into an "S" shape position, typically follows orient MAP (Braum, 1978).
Lunge:	The larva moves towards prey from the fixate position in an attempt to capture prey (Braum, 1978).

first daily feeding, larvae were arbitrarily selected from the rearing tank and placed in 2-L glass bowls containing the appropriate density of prey. For each treatment, a total of ten larvae were serially observed for two minutes each by using the focal animal technique (Altman, 1974).

After all larvae had been observed, they were returned to the general rearing tank. The daily order of observations on prey density treatments was varied systematically over the study period. The light intensity was 200 lux during observations. On observation days, standard length was measured on 12 live larvae from the rearing tank.

Larval behaviors were categorized into modal action patterns (MAPs, Barlow, 1968). During observations, the frequency and duration of the following MAPs were recorded with an event recorder: swim, turn, pause, shake, sink, orientation, fixation, and lunge. Individual MAPs are grouped into three classes: locomotory, nondirected, and foraging (Table 1).

Data analysis The effects of prey density and larval size (mm standard length) on MAP frequency and duration were analyzed with a model III ANCOVA (Zar, 1999), with size as the covariate (α=0.05; the fixed factor was prey density and size was random). Both size and age will influence the larval response to prey density. We chose to focus on the effect of size (as a surrogate for structural characteristics), rather than age (or experience) because this factor likely has a greater effect on the foraging response to prey density during this early life history stage.

The response variable represents the mean value for the 10 individual larvae per prey density for each observation day. Means for each treatment were weighted by the inverse of the standard deviation (SD) around that mean in the ANCOVA. In cases where the SD for a treatment was zero, the mean SD for that MAP (for all prey density-size combinations) was used to weight the mean for that treatment.

A linear model was used to describe the MAPs. For the turn, pause, sink and shake duration analyses, only data for the size range prior to the near decrease or disappearance of that MAP were used, in order to satisfy the assumptions of equal variance of the ANCOVA. For the swim duration analysis, only data for the size range prior to the larvae spending most (90%) of their time swimming were used. Most behavioral response variables were logarithmically transformed to satisfy model assumptions. Plots of residuals and predicted values were examined for heteroscedasticity and normality for each test, and ANCOVA assumptions were satisfied.

The orientation MAP frequency data could not be easily fitted to a linear or polynomial equation and were analyzed differently. An ANOVA was used to determine the effects of prey density on orientation frequency within the size range where orientation frequency was variable between treatments (10.5–20.8 mm). A Tukey test was then used to determine which treatment means differed (α=0.05).

We compared the lunge frequency of early (≤2 weeks) and late (≥6 weeks) stage witch flounder larvae to that of yellowtail flounder (*Pleuronectes ferrugineus*) and Atlantic cod (*Gadus morhua*) to examine differences in prey consumption rate between species. Yellowtail flounder were observed at 8000 prey/L because this prey density promotes good growth and survival for this species (Rabe and Brown, 2000). Similarly, the data used for Atlantic cod were taken from the behavioral observations conducted at 4000 prey/L presented in Puvanendran and Brown (1999). The 4000 prey/L level was the prey density that optimized growth and survival in that experiment.

Results

Experiment 1: Growth and survival

At hatching, the mean standard length of larvae was 5.62 mm (±0.12 mm SE). The standard length, dry weight, and body height of larvae did not differ between prey density

Table 2

Summary of ANOVA and ANCOVA results for growth and foraging response variables of witch flounder larvae at different prey densities (no. of prey per liter). An ANOVA was used for orient frequency within the size range 10.5–20.8 mm; for all other response variables an ANCOVA was used. Age (weeks after hatching) was used as the covariate for growth response variables, whereas size (mm SL) was used for behavioral response variables. (* denotes a significant difference at α=0.05).

MAP	Source	df	F	P
Standard length (mm)	Age	1	7041.4	<0.001*
	Prey density	2	0.85	>0.25
	Age × prey density	2	0.75	>0.25
	Error	66		
Dry weight (mg)	Age	1	2640.4	<0.001*
	Prey density	2	0.06	>0.5
	Age × prey density	2	0.21	>0.5
	Error	18		
Body height (mm)	Age	1	582.1	<0.001*
	Prey density	2	0.03	>0.5
	Age × prey density	2	0.28	>0.5
	Error	18		
Orientation frequency	Prey density	6	4.71	<0.01*
	Error	42		
Fixate frequency	Size	1	6.94	<0.05*
	Prey density	6	1.29	>0.25
	Size × prey density	6	0.52	>0.5
	Error	84		
Lunge frequency	Size	1	15.8	<0.001*
	Prey density	6	1.35	>0.25
	Size × prey density	6	0.62	>0.5
	Error	84		

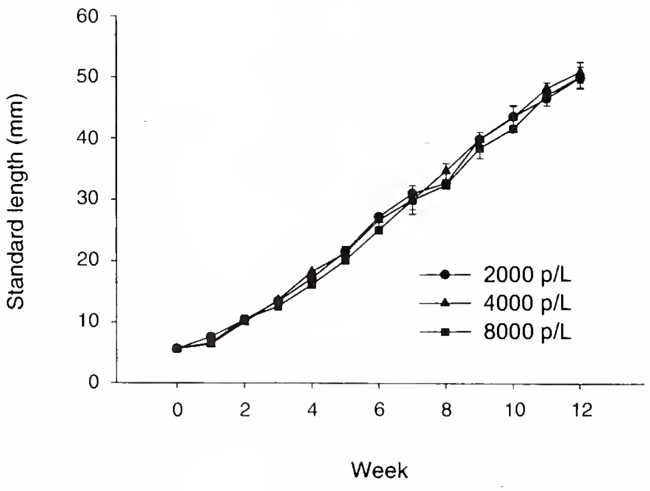

Figure 1

Mean standard length (mm) of witch flounder larvae reared at different prey densities (no. of prey per liter) over age (weeks after hatching). Values are means of the means for each replicate ±SE.

treatments (Table 2, Figs. 1 and 2). Larvae began to increase in body height around the mean size of 15 mm (Fig. 2B). The average absolute growth rate from week 0 to week 12 for all treatments was 0.53 mm/d.. The average specific growth rate (SGR) from week 0–6 was 3.68%/d and from week 0–12 was 2.61%/d.

The survival results were not corrected for fish sampled lethally (20 per tank). The survival in all treatments was similar over the course of the experiment (Table 3) and was unaffected by prey density at week 12 (ANOVA; $F_{2,3}$=2.75, P=0.210).

Experiment 2: Behavior

Larval behavior was characterized by a shift from nondirected MAPs to locomotory activities between the mean sizes of 10.5–16.2 mm (Fig. 3, A and B). The increase in locomotory activities was due to an increase in the total time spent swimming (Fig. 3A). The average duration of a swim MAP increased from 3.2 to 86.3 seconds over the study, whereas the frequency of swimming decreased significantly (Table 4). A turn MAP was used during the early stage, disappearing by the time larvae reached

10.5 mm (Fig. 3A). Pause and sink MAPs decreased when larvae reached 16.2 mm, whereas a shake MAP stopped altogether (Fig. 3B). The durations of the locomotory and nondirected MAPs changed with size (Fig. 3, A and B) but were not significantly affected by prey density (Table 4). At the end of the study there was a slight increase in pause MAPs and a concomitant decrease in the duration of swim MAPs, the result of some larvae settling during the observation periods.

Throughout the study, larvae spent between 2% and 10% of the total time observed performing foraging activities. The variation in total time spent foraging was largely due to variation in orientation duration. The duration of the fixate and lunge MAPs (<2% of total time per MAP) was relatively constant over the observation periods, whereas the duration of the orientation MAP ranged from 1% to 7% of total time (Fig. 3C).

The frequencies of the foraging behaviors were highly variable and many larvae did not forage during the observation periods. The frequency of the orientation MAP changed throughout the study period, peaking between the mean sizes of 10.5 and 20.8 mm (Fig. 4A). Within this size range, orientation frequency increased with increasing prey density. The effect of prey density on orientation frequency was significant (Table 2); the orientation frequency of larvae at 250 prey/L was significantly lower than that of larvae at 2000–16,000 prey/L within the 10.5–20.8 mm size interval (Tukey test). After 20.8 mm, the frequency of orientation was low for all sizes and treatments.

The frequencies of fixate and lunge MAPs varied from 0 to 4 per two-min observation. Larvae at higher prey densities tended to perform more fixate and lunge MAPs compared with larvae at lower prey densities (Fig. 4, B and C), although this trend was not statistically significant (Table 2). The frequencies of fixate and lunge MAPs increased significantly with increasing larval length (Fig. 4, B and C, Table 2). The average lunge frequency of early- and late-stage witch flounder larvae was lower than that of both yellowtail flounder and Atlantic cod larvae (Fig. 5).

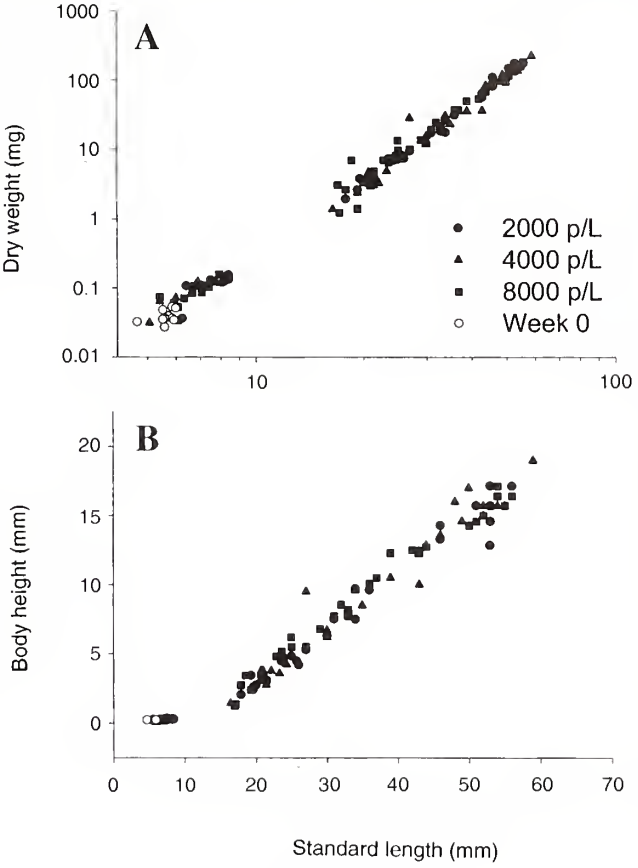

Figure 2

Relationship between standard length (mm) and (**A**) dry weight (mg; note logarithmic axis) and (**B**) body height (mm) for individual witch flounder larvae reared at different prey densities (no. of prey per liter). Symbols represent individual larvae.

Table 3

Percentage of witch flounder larvae reared at different prey densities (±SE) that survived over the experiment. Larvae sampled dead were not included in calculations.

	Prey density (no. of prey per liter)		
	2000	4000	8000
Week 2	36.15 (1.28)	30.26 (6.15)	38.20 (4.36)
Week 5	28.72 (1.54)	25.13 (5.13)	31.03 (3.85)
Week 12	14.10 (2.31)	4.62 (0.51)	8.97 (4.36)

Discussion

Witch flounder larvae grew and survived in all treatments used in our study. Our experiment is the first to examine the early growth and behavior of witch flounder larvae in relation to prey availability. Larval performance can be influenced by many factors other than prey density, including temperature (Hunter, 1981), light, (Batty, 1987; Puvanendran and Brown, 1998), prey type (Drost, 1987), and turbulence (MacKenzie and Kiørboe 1995; Browman, 1996). We used our results 1) to describe the early growth and ontogeny of the foraging behavior in this species and 2) as a preliminary step towards understanding the behavioral ecology of witch flounder larvae.

Witch flounder grew and survived equally well at each of the prey densities tested. Although the range of prey densities used in the rearing experiment (2000–8000 prey/L) was not exhaustive, these prey densities have resulted in informative differences in growth and survival for other

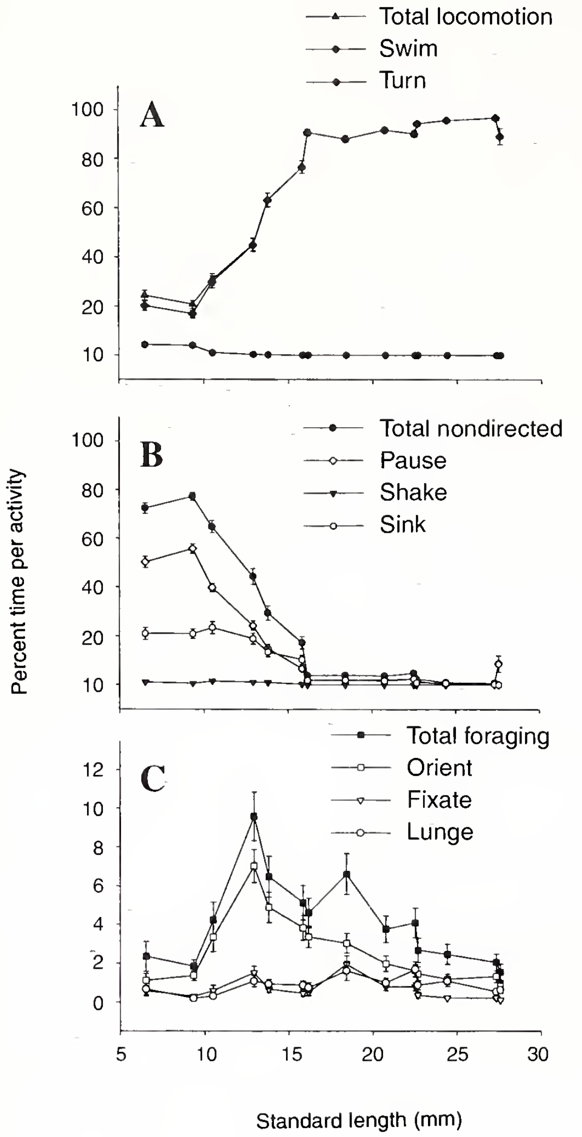

Figure 3

Mean proportion (%) of time witch flounder larvae spent performing individual (**A**) locomotory, (**B**) nondirected, and (**C**) foraging MAPs over standard length (mm) during two-minute observation periods. Values are means (n=70 larvae per length) ±SE.

North Atlantic marine fish larvae, such as Atlantic cod (Puvanendran and Brown, 1999) and redfish (*Sebastes* sp.; Laurel et al., in press) reared under similar laboratory conditions. Therefore, we anticipated that this range of prey densities would be adequate to detect differences in growth and survival of witch flounder in relation to prey density. Furthermore, as shown in experiment 2, the lunge frequency of witch flounder was not significantly affected by prey availability, which would be expected if low prey densities (<2000 prey/L) were to reduce consumption and subsequent growth and survival.

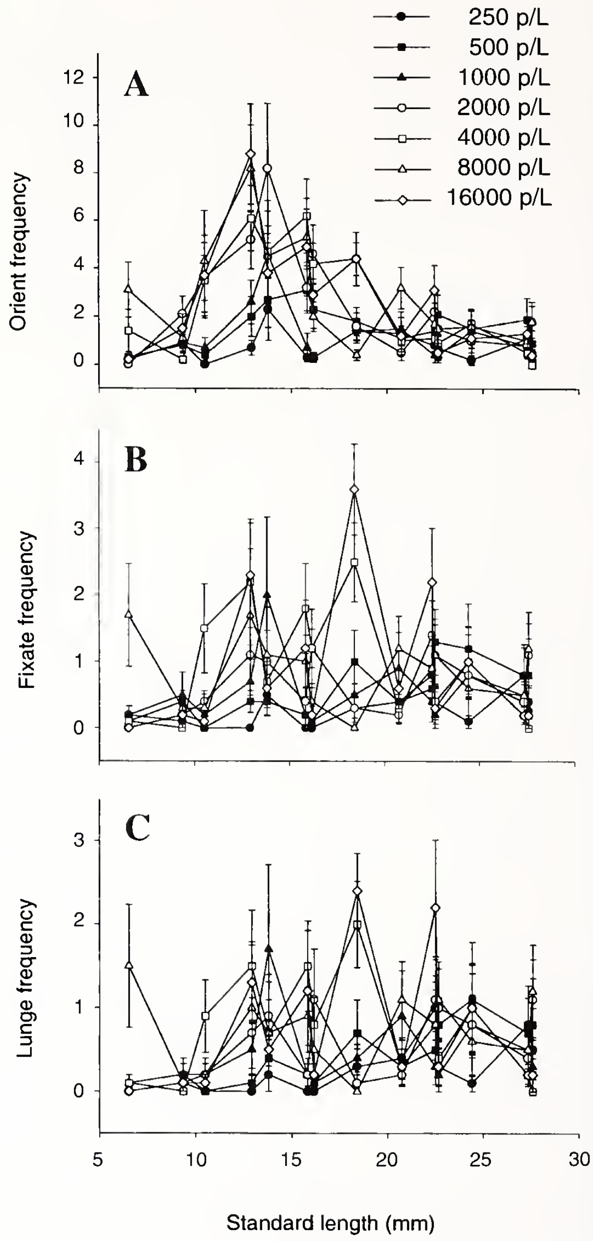

Figure 4

Frequency of (**A**) orient, (**B**) fixate, and (**C**) lunge MAPs of witch flounder larvae at different prey densities (no. of prey per liter) during two-minute observation periods over standard length (mm). Values are means (n=10) ±SE.

Ontogeny of behavior

Witch flounder search strategy for prey is interesting because it appeared to change from a saltatory to a cruise strategy (see O'Brien et al., 1990; Browman and O'Brien, 1992) during the study period. When larvae were less than 10 mm, foraging included many turns and brief periods of swimming that served as repositioning acts. By the time larvae reached an average size of 16.2 mm, most of their

Table 4

Summary of ANCOVA results for locomotory and nondirected MAPs of witch flounder larvae at different prey densities (no. of prey per liter). Each model was run until the larval size indicated in parentheses to satisfy model assumptions. (* denotes a significant difference at α=0.05).

MAP	Source	df	*F*	*P*
Swim frequency (20.8 mm)	Size	1	32.1	<0.001*
	Prey density	6	0.78	>0.25
	Size × prey density	6	0.42	>0.5
	Error	49		
Swim duration (20.8 mm)	Size	1	251.4	<0.001*
	Prey density	6	1.76	>0.25
	Size × prey density	6	0.17	>0.5
	Error	49		
Turn duration (13.8 mm)	Size	1	56.5	<0.001*
	Prey density	6	1.34	>0.25
	Size × prey density	6	0.82	>0.5
	Error	21		
Pause duration (20.8 mm)	Size	1	88.4	<0.001*
	Prey density	6	1.82	>0.25
	Size × prey density	6	0.26	>0.5
	Error	49		
Sink duration (18.4 mm)	Size	1	79.8	<0.001*
	Prey density	6	0.54	>0.5
	Size × prey density	6	1.35	>0.5
	Error	42		
Shake duration (16.2 mm)	Size	1	13.0	<0.001
	Prey density	6	1.13	>0.25
	Size × prey density	6	0.25	>0.5
	Error	35		

time was spent swimming, which was typically interrupted only by foraging events. During the mean size interval of 10.5–16.2 mm, the turn and shake MAPs disappeared and the frequency of pause and sink MAPs decreased. These behavioral changes were likely related to the increase in larval body height, accompanied by a substantial increase in finfold height that occurs during this time.

The nature of the nondirected MAPs shake, sink, and pause is not straightforward. Sinking has been reported in other species, such as the snapper (*Pagrus auratus*) and, like the pause MAP, has been interpreted as a resting behavior. In snapper, it occurs in yolksac larvae and in feeding larvae during night-time periods of inactivity (Pankhurst et al., 1991). Sinking is typically observed only in the early stages of other species such as the black sea bream, *Acanthopagrus schlegeli* (Fukuhara, 1987). However, Kawamura and Ishida (1985) noted that sinking occurs in both yolksac larvae and larger feeding larvae of the flounder *Paralichthys olivaceus* immediately after attacking a prey item. Observations of sinking in later-stage witch flounder larvae were not related to feeding events; the persistence of these behaviors in witch flounder was likely the result of some smaller, slower-growing individuals having been included in our observations.

Witch flounder, like other species (Holling, 1965; Houde and Schekter, 1980; Werner and Blaxter, 1980; Puvanendran and Brown, 1999), demonstrated increased foraging behavior with prey density. However, the orient MAP was the only foraging MAP statistically affected by prey density. The change in orientation frequency with size is interesting because this MAP was affected only by prey density within a limited size range. The initial low frequency of orient MAPs followed by an increase associated with greater larval size can be explained by changes in swimming speeds and encounter rates (Mittelbach, 1981). However, the decrease in orient frequency across treatments later in the study period is puzzling because larger larvae are generally competent swimmers (Rosenthal and Hempel, 1971; Laurence, 1972; Houde and Schekter, 1980) and are expected to exhibit a high prey encounter rate. This decrease in orient frequency may be due to improved foraging ability associated with greater visual acuity. Miller et al. (1993) showed that the visual angle—the smallest angle at which a stimulus may subtend the eye and remain resolvable (Neave, 1984)—decreases during the development of three species of fish larvae. Thus, the eye develops such that larvae can likely detect prey items in their periphery without turning the head and orienting themselves toward them.

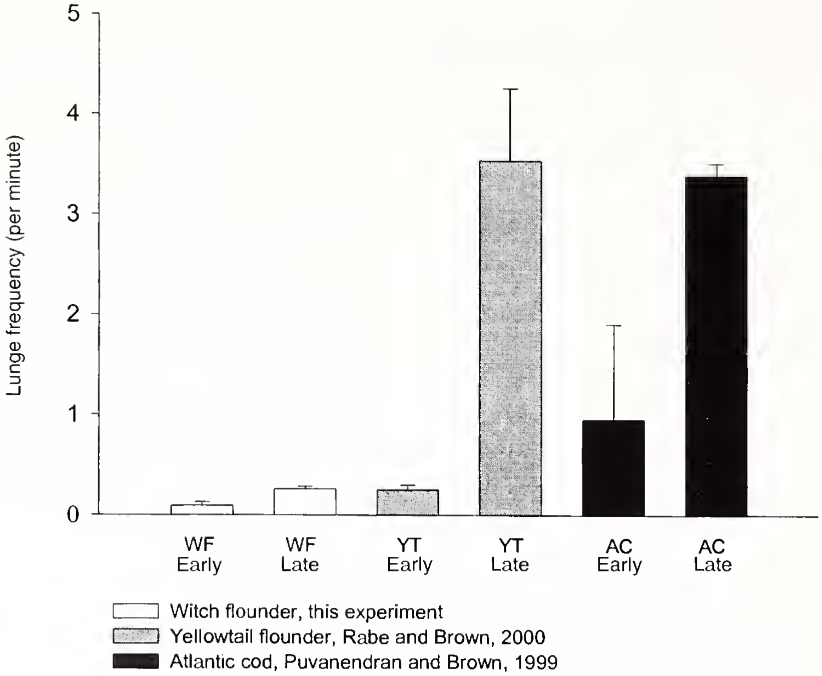

Figure 5

Average lunge frequency per minute for early (≤2 weeks) and late stage (≥6 weeks) witch flounder (WF), yellowtail flounder (YT), and Atlantic cod larvae (AT). See text for details.

Behavioral ecology

The main finding of our study was that witch flounder are not affected by changes in prey availability in the same manner as other species of larvae observed under similar laboratory conditions. The typical pattern among fish larvae—that they increase their foraging behavior and prey consumption rate with increased prey density (Houde and Schekter, 1980)—was supported by our study. However, the results are unusual in that the effects of prey density on foraging behavior were not as strong as results that have been reported for other species.

The ecological implications of these results can be illustrated by a comparison of the growth and behavior of witch flounder to other northwest Atlantic species observed under similar laboratory conditions. In rearing experiments on Atlantic cod larvae, Puvanendran and Brown (1999) found that cod have specific requirements for high prey densities. Larval survival, growth rate (0.20 mm/d), and condition were highest when larvae were reared at a prey density of 4000 prey/L. Furthermore, in the same experiment, the lunge frequency (an indicator of consumption rate) of cod larvae increased from nearly 1 to 3.5 prey items per minute over the six-week study period (Fig. 5; Puvanendran and Brown, 1999). A marked increase in lunge frequency with age is also seen in yellowtail flounder, another north Atlantic pleuronectiform, raised under similar conditions (Fig. 5; Rabe and Brown, 2000). In that study, the growth rate of yellowtail flounder was 0.34 mm/d.

Not only is the foraging behavior of witch flounder relatively unaffected by variation in prey density, but its lunge frequency is much lower, suggesting that it may have a lower consumption rate and therefore lower prey requirements compared with those of other species. The lack of a significant effect of prey density on the foraging of witch flounder is not solely a result of the larger larval size of this species. Redfish (*Sebastes*) are relatively large at extrusion (6–8.9 mm; Penny and Evans, 1985) and their foraging behavior is affected by variations in prey availability (Laurel et al., in press).

It is remarkable that the growth rate of witch flounder is faster than that of cod and yellowtail flounder, especially given the possible lower prey consumption rate of witch flounder. Two potential mechanisms that may explain this phenomenon are high assimilation efficiency and low metabolic requirements. Witch flounder are relatively large at hatching and for most of the study period were larger than other species of similar age. This size difference alone implies that its digestive system is larger, more developed, and more efficient (Govoni et al., 1986; Klumpp and von Westernhagen, 1986). Both yellowtail flounder and cod are more active and swim faster than witch flounder. Higher activity imparts a greater need for prey, which results in larvae being more susceptible to starvation in the absence of prey (Hunter, 1981).

The ecological significance of the lack of an effect of prey density on foraging, growth, and survival found in our study may be a reflection of the life history of witch flounder. This species has an extended larval period and is committed to being in the water column much longer than other species (Bigelow and Schroeder, 1953). During this extended larval period, witch flounder larvae will likely encounter periods of high or low plankton availability, or both. The abundance of zooplankton prey for fish larvae can vary over four orders of magnitude during the year, typically reaching a peak in the warmer months and decreasing dramatically in the winter (Myers et al., 1994). Therefore, witch flounder larvae must be able to cope with this variation in prey availability to survive. This condition requires a different strategy from that of other marine fish species that have shorter larval periods and that likely rely on a "match" of spawning with plankton production to promote larval survival (Cushing, 1972). In the scope of the life history evolution of witch flounder, the long larval period and large size at metamorphosis may be a strategy to cope with intense postsettlement, size-dependent competition.

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Lack of divergence between 16S mtDNA sequences of the swimming crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela*

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Abstract—Lake Maracaibo, a large Venezuelan estuarine lagoon, is reportedly inhabited by three species of the genus *Callinectes* Stimpson, 1860 that are important to local fisheries: *C. sapidus* Rathbun, 1896, *C. bocourti* A. Milne Edwards, 1879, and *C. maracaiboensis* Taissoun, 1969. *Callinectes maracaiboensis*, originally described from Lake Maracaibo and assumed endemic to those waters, has recently been reported from other Caribbean localities and Brazil. However, because characters separating it from the morphologically similar *C. bocourti* are noted to be vague, we have compared these species and several congeners by molecular methods. Among our specimens from Lake Maracaibo and other parts of the Venezuelan coast, those assignable to *C. bocourti* and *C. maracaiboensis* on the basis of putatively diagnostic characters in coloration and structural characteristics do not differ in their 16S mtDNA sequences. These molecular results and our re-examination of supposed morphological differences between these species suggest that *C. maracaiboensis* is a junior synonym of *C. bocourti*, which varies markedly in minor features of coloration and structural characteristics. Genetic relationships of this species to other species of swimming crabs of the genus *Callinectes* are also explored and presented as phylogenetic trees.

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Swimming crabs of the genus *Callinectes* Stimpson, 1860 are widely distributed throughout the American tropics and subtropics, where many species are exploited commercially (Norse, 1977; Williams, 1984). Members of this genus play key trophic roles in coastal habitats that range from sandy-mud bottoms to seagrass meadows (Arnold, 1984; Orth and van Montfrans, 1987; Wilson et al., 1987; Lin, 1991). Of the nine species of *Callinectes* from the tropical western Atlantic, seven have been reported from western Venezuela (Rodríguez, 1980; Williams, 1984; Carmona-Suárez and Conde, 1996). However, knowledge of these Venezuelan populations remains inadequate, despite the importance of several species in both large-scale commercial harvests and artisanal fisheries of coastal villages in the area (Oesterling and Petrocci, 1995; Ferrer-Montaño, 1997; Conde and Rodríguez, 1999). Persistent difficulty in identifying mixed samples of these species hampers understanding of their distribution, abundance, and population dynamics, which are essential to fishery-management decisions. In particular, consistent morphological distinction of the commonly encountered *C. bocourti* from *C. maracaiboensis* cannot be achieved with confidence.

Initially described as endemic from Lake Maracaibo, *C. maracaiboensis* has been reported from other Venezuelan waters, as well as from sites in Jamaica, Curaçao, Colombia, and Brazil (Norse, 1977; Carmona-Suárez and Conde, 1996; Sankarankutty et al., 1999). Although detailed descriptions have been provided by Taissoun (1969, 1972) and Williams (1974) to diagnose *C. maracaiboensis*, the same authors have repeatedly emphasized its resemblance to *C. bocourti*. Morphological distinction of the two species is based upon postulated defining characters of the frontal teeth, direction and form of the anterolateral spines, convexity of the carapace, surface granulation patterns, and varied morphometric measurements (Taissoun 1969, 1972). However, neither these characters nor additional ones proposed by other authors (Williams, 1974; Rodríguez, 1980) have proven to be of consistent use in separating the species. Carapacial color and granulation vary widely, and the outer frontal teeth of many specimens cannot be classified as a definitive triangular (*C. maracaiboensis*) or obtuse (*C. bocourti*) shape. These concerns have led some authors to report only possible co-occurrence of *C. maracaiboensis* in their samples of *C. bocourti*, as in records from Puerto Rico (Buchanan and Stoner, 1988).

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Recently, molecular markers have proven particularly valuable in decapod crustacean systematics. In addition to reconstructing phylogenetic relationships, molecular sequences are of value in recognizing questionable species separations (e.g. Geller et al., 1997; Sarver et al., 1998; Schneider-Broussard et al., 1998; Schubart et al., 1998; Schubart et al., in press). In our study, we used sequences of a mitochondrial gene (16S rRNA) to examine genetic differentiation between the morphologically similar species *C. bocourti* and *C. maracaiboensis* and to compare these sequences to those of other swimming crabs of the genus *Callinectes* in a phylogenetic analysis.

Materials and methods

Swimming crabs were caught by hand in shallows of the Golfete de Cuare (68°37′W, 10°06′N, State of Falcón, Venezuela) in November–December 1998 and March 1999, and in the shallows of El Moján, Lago de Maracaibo (71°39′W, 10°58′N, State of Zulia, Venezuela) in October 1999. Crabs were transported to the Instituto Venezolano de Investigaciones Científicas, Caracas, in liquid nitrogen and then stored in a –70°C freezer. Two walking legs and one swimming leg were separated from each specimen, preserved individually in 85% ethanol, and shipped to the University of Louisiana, Lafayette (U.S.A.), for molecular analyses.

Specimens were assigned to *Callinectes bocourti* or *C. maracaiboensis* according to morphological characters provided by Taissoun (1969, 1972), Williams (1974, 1978), Fischer (1978), and Rodríguez (1980). Eight specimens of *C. bocourti* and six of *C. maracaiboensis* from Venezuela were used for the molecular analysis. In addition, a short fragment (~300 base pairs) of 16S mtDNA was obtained with an internal primer for one formalin-preserved specimen of *C. bocourti* from Colombia. These voucher specimens, lacking limbs (which had been removed for analysis), were cataloged in collections of the Laboratorio de Ecología y Genética de Poblaciones, Instituto Venezolano de Investigaciones Científicas (IVIC), and in the University of Louisiana Zoological Collection (Table 1). For outgroup comparisons, specimens of *C. sapidus*, *C. ornatus* Ordway, 1863, *C. danae* Smith, 1869, and *Portunus ordwayi* (Stimpson, 1860) were sequenced (Table 1).

Genomic DNA was isolated from the muscle tissue of one walking leg with a phenol-chloroform extraction (Kocher et al., 1989). Selective amplification of a 585-basepair (bp) product (547 bp excluding primer regions) from the mitochondrial 16S rRNA gene was carried out by a polymerase-chain-reaction (PCR) (35–40 cycles: 1 min at 94°C, 1 min at 55°C, 2 min at 72°C (denaturing, annealing, and extension temperatures, respectively) with primers 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′), 16Sbr (5′-CCGGTCTGAACTCAGATCACGT-3′), 1472 (5′-AGATAGAAACCAACCTGG-3′), and 16L15 (5′-GACGATAAGACCCTATAAAGCTT-3′) (for references to primers see Schubart et al., 2000). 16L15 is an internal primer and, in combination with 16Sbr, was used for partial amplification of the formalin-preserved specimen. PCR products were purified with Microcon-100® filters (Millipore Corp., Bedford, MA) and sequenced with the ABI BigDye® Terminator Mix (PE Biosystems, Wellesley, MA) in an ABI Prism 310 Genetic Analyzer® (Applied Biosystems, Foster City, CA). Sequences were manually aligned with the multisequence-editing program ESEE (Cabot and Beckenbach, 1989). New sequences were accessioned to the European Molecular Biology Laboratory (EMBL) genomic library (see Table 1). Sequences available online (by electronic database accession numbers that follow) were obtained for "*Callinectes sapidus*" (U75267), *C. ornatus* (U75268), *C. similis* (U75269), and *C. sapidus* (AJ130813).

Sequence divergence was analyzed by using Kimura 2-parameter distances, UPGMA cluster analysis, and neighbor-joining (NJ) distance analysis (Saitou and Nei, 1987) with the program MEGA (Kumar et al. 1993). Statistical significance of groups within inferred trees was evaluated by the interior branch method (Rzhetsky and Nei, 1992). As a second phylogenetic method, a maximum parsimony (MP) analysis was carried out with a heuristic search and random sequence addition (tree-bisection and reconnection as branch-swapping option) and by omission of gaps with the program PAUP (Swofford, 1993). Significance levels were evaluated with the same software and the bootstrap method with 2000 replicates.

Results

Sequencing of 15 specimens of *Callinectes bocourti* and *C. maracaiboensis* from Golfete de Cuare and El Moján (both Venezuela) and the Río Sinú estuary (Colombia) revealed the existence of seven different haplotypes. Two haplotypes (ht-1 and ht-5) clearly predominated (together 64.3%) and were found in both species (ht-1 in two *Callinectes bocourti* and three *C. maracaiboensis* from Golfete de Cuare, ht-5 in three *C. bocourti* and one *C. maracaiboensis* from El Moján). The other five haplotypes (ht-2, ht-3, ht-4, ht-6, ht-7) each differed from ht-1 or ht-5 in not more than two positions and were found in only single individuals (Fig. 1, Table 2). Consequently there is not a single diagnostic molecular character in our sequenced unit of 16S mtDNA that would discriminate between the two species of swimming crabs. On the other hand, haplotype distributions differed between the two sampled populations; ht-1 is found in only the Golfete de Cuare (~550 km east of Lake Maracaibo) and ht-5 is restricted to El Moján (within Lake Maracaibo). Comparison of the

Table 1

Swimming crabs used in sequencing of 16S mtDNA and subsequent phylogenetic analyses. IVIC-LEGP = Laboratorio de Ecología y Genética de Poblaciones, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; ULLZ = University of Louisiana Zoological Collections, Lafayette, U.S.A.; specimens bearing numbers for both collections are archived in ULLZ; EMBL = European Molecular Biology Laboratory.

Species	Locality of collection	Catalog number	EMBL accession no.
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-40 = ULLZ 4181	AJ298171
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-70	AJ298182
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-71	AJ298172
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-72	AJ298173
Callinectes maracaiboensis	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-1	AJ298177
Callinectes maracaiboensis	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-6	AJ298183
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEPG-C-30 = ULLZ 4180	AJ298170
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-109	AJ298174
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-112	AJ298175
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-113	AJ298176
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-2	AJ298181
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-3	AJ298178
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-4	AJ298179
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-5	AJ298180
Callinectes bocourti	Colombia: Río Sinú	ULLZ 4186	AJ298169
Callinectes sapidus	Florida: Fort Pierce	ULLZ 3766	AJ298189
Callinectes sapidus	Venezuela: Zulia: El Moján	ULLZ 4188	AJ298190
Callinectes ornatus	Venezuela: Falcón: La Vela de Coro	IVIC-LEGP-LV-CO-1	AJ298187
Callinectes ornatus	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-CO-3	AJ298188
Callinectes ornatus	Brazil: Ensenada de Ubatuba	ULLZ 4178	AJ298186
Callinectes danae	Brazil: Ensenada de Ubatuba	ULLZ 4179	AJ298185
Callinectes danae	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-1	AJ298184
Portunus ordwayi	Venezuela: Falcón: La Vela de Coro	IVIC-LEGP-LV-9	AJ298191

Table 2

Percent (uncorrected) genetic divergence and number of differences between 547 base pairs of 16S mtDNA among 7 haplotypes (ht) corresponding to 14 specimens of *Callinectes bocourti* and *C. maracaiboensis* from Venezuela (s: transition, v: transversion, i: indel). Catalog numbers shown below correspond to IVIC-LEGP specimens listed in Table 1.

	ht-1	ht-2	ht-3	ht-4	ht-5	ht-6	ht-7
	C-30 C-40 C-71 C-72 C-109	C-70	C-112	C-113	MZ-1 MZ-3 MZ-4 MZ-5	MZ-2	MZ-6
ht-1	—	0.18	0.18	0.18	0.18	0.55	0.18
ht-2	1v	—	0.37	0.37	0.37	0.73	0.37
ht-3	1s	1s, 1v	—	0.37	0.37	0.73	0.37
ht-4	1s	1s, 1v	2s	—	0.37	0.73	0.37
ht-5	1s	1s, 1v	2s	2s	—	0.37	0.37
ht-6	2s, 1i	2s, 1i, 1v	3s, 1i	3s, 1i	1s, 1i	—	0.73
ht-7	1s	1s,1v	2s	2s	2s	3s, 1i	—

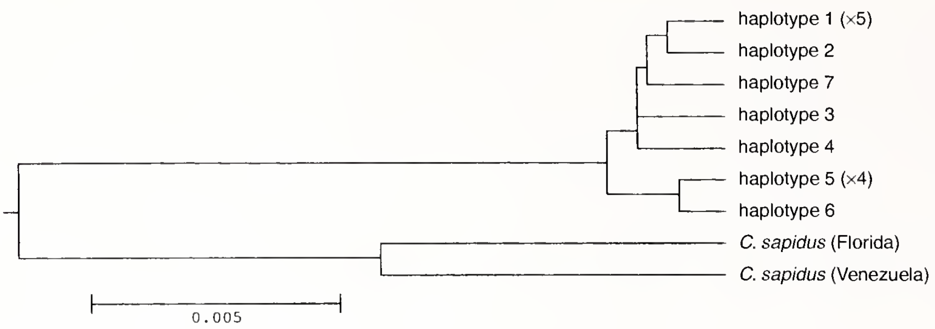

Figure 1

UPGMA (unweighted pair-group method with arithmetic averaging) analysis of Kimura 2-parameter genetic distances based on 547 base pairs of 16S mtDNA among seven haplotyopes of *Callinectes maracaiboensis* and *C. bocourti* (see Table 2 for species designation) corresponding to 14 specimens from Golfete de Cuare (haplotypes 1–4) and El Moján (haplotypes 5–7) (both Venezuela) and two populations of *C. sapidus.*

294 base pairs obtained for one *Callinectes bocourti* from Colombia did not reveal any difference from ht-1, ht-3, or ht-5, suggesting that genetic homogeneity in these species can be expected in other Caribbean localities.

Comparing sequences from *C. danae*, *C. ornatus*, *C. sapidus*, and *Portunus ordwayi* with those of *C. bocourti* and *C. maracaiboensis* allowed us to postulate phylogenetic relationships among these selected portunid taxa (Fig. 2). Marked genetic differences (Table 3) characterized all intra- and interspecific separations within the genus *Callinectes*, except in pairings of *C. bocourti* and *C. maracaiboensis.*

Discussion

Comparison of 15 sequences of the 16S mtDNA of *Callinectes maracaiboensis* and *C. bocourti* from within and outside Lake Maracaibo revealed no consistent differences between these two species of swimming crabs. These results support observations from previous studies in which morphological characters have proven unreliable in separating these two species. Rather than constituting a separate species, *C. maracaiboensis* would appear from these findings to represent only a phenotypic extreme of *C. bocourti*, a species of known morphological plasticity (Williams, 1974).

We are highly confident that our sequenced samples of *C. maracaiboensis* represent the population originally assigned to that species. In addition to assuring that our samples were topotypic, we confirmed individual specimen identities by direct comparisons with deposited paratypes of Taissoun (IVIC-LEGP 425). However, we cannot make similar claims for the analyzed materials of *C. bocourti.* Although published records (Williams, 1984) indicate that *C. bocourti* is widely distributed (from Jamaica and Belize to Brazil, occasionally northward to Florida, Mississippi, and North Carolina), the type locality is southern Belize. To date, topotypic materials of *C. bocourti* have not been available to us for use in either our sequencing or morphological comparisons with Venezuelan material; such comparisons should, however, ultimately be undertaken to firmly anchor the synonymy we have proposed. Among specimens that we would presently assign to *C. bocourti* on the basis of structural features, one from the Río Sinú of Colombia shared complete identity in almost 300 base pairs of mtDNA with the most common haplotypes from Venezuela. This finding at the very least suggests a large overall range for materials that we refer to as *C. bocourti* and a relative homogeneity among forms that we have assigned to this name on the basis of structural characteristics, even over large geographic distances.

The reported phylogenetic relationships and genetic distances (Fig. 1) reflect a low level of genetic divergence among the seven haplotypes of *C. maracaiboensis* and *C. bocourti*, especially when compared with sequences of two populations of *C. sapidus.* The tree resulting from the phylogenetic analysis (Fig. 2) also suggests that *C. maracaiboensis* and *C. bocourti* are closer to *C. sapidus* than to *C. ornatus*, *C. similis*, and *C. danae*, which is in accord with preliminary findings of Norse and Fox-Norse (1979). However, our results must also be regarded as preliminary because other species of *Callinectes* should be sequenced before we can claim full understanding of sister-species relationships. Published studies to date that have attempted to separate questionable brachyuran species on the basis of 16S mtDNA have consistently reported at least a few nucleotide differences that are diagnostic for the species in question (Geller et al., 1997; Schneider-Broussard et al., 1998; Schubart et al. 1998; Schubart et al., 2000). Only the Mediterranean species *Brachynotus sexdentatus* and *B. gemmellari* may constitute an exception to this pattern because they are identical in the sequenced fragment of 16S mtDNA (Schubart et al., in press).

Comparison of our sequences with those from a previous study (Geller et al., 1997) revealed important, apparently intraspecific differences between specimens of *C. ornatus* as well as between specimens of *C. sapidus.* Genetic

Table 3

Percent genetic divergence (uncorrected; excluding indels) between 553 base pairs of 16S mtDNA among species of *Callinectes* and the outgroup *Portunus ordwayi*, as used for the phylogeny in Figure 2 (1=*C. similis* (U75269); 2="*C. sapidus*" (U75267); 3=*C. sapidus* (FL); 4=*C. sapidus* (Venezuela); 5=*C. bocourti* (ht-1); 6=*C. bocourti* (ht-5); 7=*C. danae* (Venezuela); 8=*C. danae* (Brazil); 9=*C. ornatus* (U75268); 10=*C. ornatus* (Venezuela CO-1); 11=*C. ornatus* (Venezuela CO-3); 12=*C. ornatus* (Brazil); 13=*P. ordwayi*).

	2	3	4	5	6	7	8	9	10	11	12	13
1	0.18	5.61	5.79	6.33	6.15	0.54	0.54	2.53	3.07	3.07	2.89	13.02
2		5.06	5.24	5.79	5.61	0.54	0.54	2.53	3.07	3.07	2.89	12.12
3			1.27	2.53	2.35	5.79	5.79	5.97	6.87	6.87	6.69	13.92
4				2.71	2.53	6.15	6.15	6.15	7.05	7.05	7.05	13.92
5					0.18	6.51	6.51	6.33	6.51	6.51	6.33	14.10
6						6.51	6.51	6.15	6.33	6.33	6.33	14.29
7							0.00	2.71	3.62	3.62	3.44	13.92
8								2.71	3.62	3.62	3.44	13.92
9									1.63	1.63	1.45	12.66
10										0.36	0.18	12.84
11											0.18	13.02
12												13.38

differences between Geller et al.'s (1997) specimen from North Carolina (U75268) and three specimens of *C. ornatus* from South America can be attributed to geographical distance. In the case of *C. sapidus*, however, differences clearly exceed a level that could be interpreted as ancestral polymorphism or biogeographical variation. Although our new sequence of *C. sapidus* from Florida perfectly matches one previously registered for a specimen from Louisiana (AJ130813), and both of these show limited divergence from a Venezuelan specimen, another sequence previously reported for *C. sapidus* (U75267) by Geller et al. (1997) differs markedly from the aforementioned set. The phylogeny of all 16S sequences presently available for *Callinectes* (Fig. 2) shows that the sequence reported for "*C. sapidus*" by Geller et al. (1997) instead pairs closely with *C. similis*, a grouping supported by high bootstrap values. Because no morphological voucher specimens appear to exist for this sequenced specimen,[1] we must conclude that the reported sequence was based upon a misidentified specimen of *C. similis*.

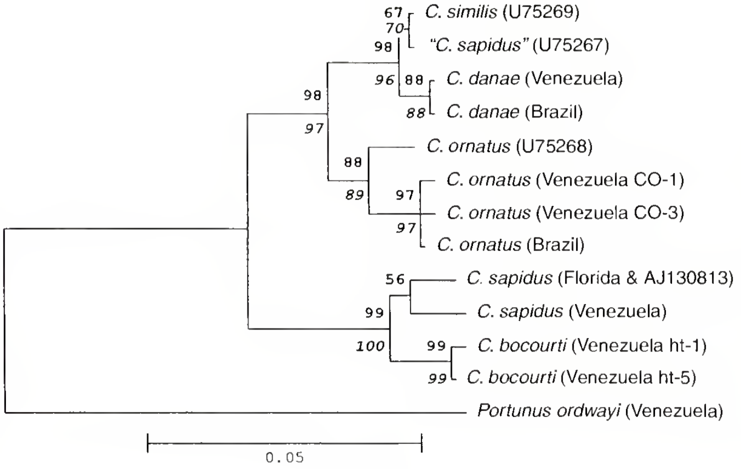

Figure 2

Phylogenetic relationships of five species of *Callinectes* based on 553 base pairs of 16S mtDNA. Tree topology is based on neighbor-joining (NJ) analysis with Kimura 2-parameter genetic distances. Sequences accessed online for *C. similis*, putative "*C. sapidus*," and *C. ornatus*, and *C. sapidus*, are indicated solely by electronic database accession numbers. Analyses for Venezuelan *C. ornatus* include specimens from adjacent localities (see Table 1); those for *C. bocourti* include haplotypes (ht) 1 and 5 (see Table 2 and Fig. 1). Confidence values ≥50 were obtained with the interior branch method for NJ distance analysis (upper values) and by maximum parsimony analysis after 2000 bootstrap replicates (lower values, in Italics).

Differences in color of some of the Brazilian specimens (see Sankarankutty et al., 1999) could indicate that two or more species might be involved in the *C. bocourti* complex, regardless of the synonymy that we have herein proposed. However, color differences should be judged with precaution, given the known high variability of this feature in *C. bocourti* (see Williams, 1984). Our recent color photography of specimens from Colombia, Venezuela, and

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[1] Geller, J. 1999. Personal commun. Moss Landing Marine Laboratories, Moss Landing, CA 95039.

Florida has revealed marked variations; dorsal surfaces of the carapace of some specimens were predominantly olive green, those in other specimens were largely rust brown or rust patterned on green, and in yet others a rust pattern on a background of almost totally blue.

As a result of our molecular analyses, especially in the absence of consistent characters to distinguish *C. maracaiboensis* on the basis of color or structural characteristics, we must conclude that Lake Maracaibo and adjacent Venezuelan waters are inhabited by populations assignable to *C. sapidus* and by only one other species of the genus, *C. bocourti*. It appears that these are in turn the target species of a largely unregulated crab fishery which represented more than U.S. $6 million in exports to the United States in 1992 (Oesterling and Petrocci, 1995), even though it has declined since 1989 in probable response to overharvesting. As maximum sustainable yield has likely been achieved, these fisheries, which have been proposed for Miller's phase II management (resource-mapping, gear related regulations), should in the near future proceed to phase III (basic biological studies, long-term management) (Miller, 1999). This phase can be undertaken only with a clear understanding of the genetic entities upon which the fishery is based.

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Feeding habits, prey fields, and potential competition of young-of-the-year walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, 1994–1995

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Abstract—Diets of young-of-the-year (YOY) walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) were compared between seasons (summer and autumn), years (autumn), and allopatric and sympatric fish aggregations (autumn) in Prince William Sound (PWS), Alaska. Fish were collected principally by mid-water trawl 20 July–12 August 1995, 5–14 October 1995, and 7–13 November 1994. Prey fields were assessed from zooplankton samples in 1995.

During the summer, the principal prey of allopatric pollock and herring was small calanoids and diet overlap was high (R_0>0.76). During the autumn, diets were composed of large calanoids, larvaceans, and euphausiids. Diet overlap between sympatric species was greater in November 1994 (R_0≤0.94) than in October 1995 (R_0≤0.69). The seasonal diet shift to larger prey coincided with larger fish size and with decreased abundance and proportions of the principal zooplankter, small calanoids, and increased abundance and proportions of large calanoids and larvaceans in zooplankton tows. However, feeding decreased in autumn, compared with summer, especially for herring. Sympatric fish had higher rates of nonfeeding than allopatric fish, and subtle differences in prey selection existed between the aggregations, but sampling variation could explain these feeding differences.

The similarity in diets of YOY pollock and herring indicate the potential for competition. These species are important to commercial fisheries and as forage for marine birds and mammals. An understanding of their trophic interactions could help to explain shifts in fish community structure and bird predation. If sympatry increases as prey resources decline, competition in autumn may be particularly important in regulating populations.

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Walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) are forage fish that inhabit the northeastern Pacific Ocean rim. Both species are important components of marine bird, mammal, and fish diets, both support important commercial fisheries in the Gulf of Alaska (GOA), and historical data show dramatic variability in both their populations (Springer, 1992; Bechtol, 1997; Springer and Speckman, 1997; Anderson and Piatt, 1999). Young-of-the-year (YOY) walleye pollock and YOY Pacific herring are found at the same locations and depths during at least part of the year (Brodeur and Wilson, 1996a; Willette et al., 1997; Stokesbury et al., 2000) and both consume zooplankton as their primary prey[1,2] (Willette et al., 1997; Foy and Norcross, 1999a, 1999b). Because of these similarities and because the frequency and nature of their interactions may change as fish population structure shifts, we investigated the potential for feeding competition between these species.

Several recent studies have found that the species composition of forage fish populations in the GOA and Prince William Sound (PWS) has changed dramatically (Bechtol, 1997; Kuletz et al., 1997; Anderson and Piatt, 1999). Short-term population changes were attributed to the *Exxon Valdez* oil spill in March 1989 (Brown et al., 1996a; Kuletz, 1996; Oakley and Kuletz, 1996; Kuletz et al.,

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[2] Boldt, J. L. 1997. Condition and distribution of forage fish in Prince William Sound, Alaska. Unpubl. M.S. thesis, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801, 155 p.

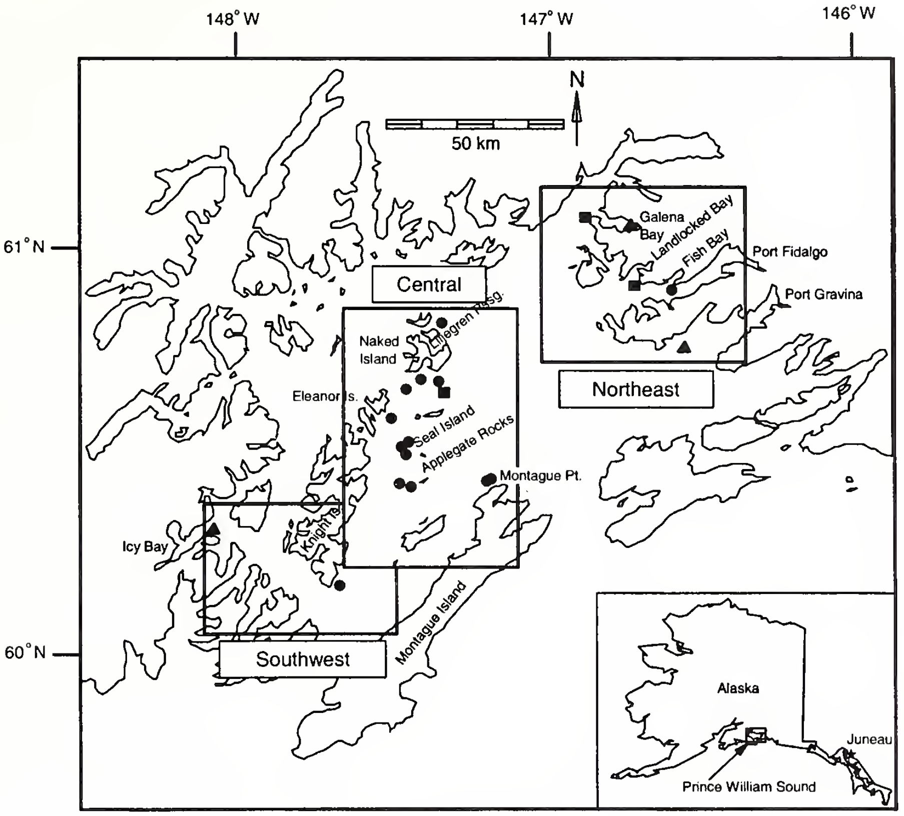

Figure 1

Sampling regions and stations for YOY walleye pollock and Pacific herring diet samples collected in Prince William Sound, Alaska. Circles: July–August 1995; squares: October 1995; triangles: November 1994.

1997), but researchers believe that long-term changes began with a climate regime shift prior to the oil spill (Bailey et al., 1995; Piatt and Anderson, 1996; Anderson and Piatt, 1999). For example, the number of walleye pollock and other demersal fish increased at the same time that the other taxa decreased (Anderson and Piatt, 1999), the PWS spawning population of Pacific herring declined by 75% by 1993 (Brown et al., 1996b), and fish biomass of PWS decreased by 50% (Piatt and Anderson, 1996). Concomitant decreases in populations of marine birds and mammals in PWS may be related to these shifts in the composition and abundance of forage fish prey (Oakley and Kuletz, 1996; Piatt and Anderson, 1996; Iverson et al., 1997). Apparently, fewer high-quality forage fish have been available, and the species composition has changed to one in which the predominant fish taxa are less energetically valuable to marine piscivores (Piatt and Anderson, 1996; Anthony and Roby, 1997; Anderson and Piatt, 1999; Payne et al., 1999). The potential for such shifts to cascade throughout marine food webs (Livingston, 1993; Springer and Speckman, 1997) is an important reason to understand the trophic interactions of forage fish.

This reports stems from the Alaska Predator Ecosystem Experiment (APEX), a multidisciplinary study that attempted to link current knowledge about the forage fish of PWS with their seabird predator populations. We describe differences in the feeding habits of YOY walleye pollock and YOY Pacific herring caught in summer and autumn in PWS and compare feeding attributes of fish caught in allopatric (single species) and sympatric (co-occurring, multispecies) aggregations in autumn to support the hypothesis that the presence of potential competitors may induce changes in feeding habits. We compare fish size, zooplankton prey fields, fish feeding habits, prey selection, food quantity, and diet overlap of these species.

Materials and methods

Field methods

Fish stomach and zooplankton samples were collected during APEX forage fish population surveys in central, northeastern, and southwestern PWS[3,4,5] (Fig. 1). In a pilot study in 1994, we sampled from 7 to 13 November aboard the Alaska Department of Fish and Game RV *Medeia*; in 1995, we sampled from 20 July to 12 August aboard the charter FV *Caravelle,* and from 5 to 14 October aboard the RV *Medeia*. Surveys were conducted offshore along a grid of parallel transects spaced at two-mile intervals and ending as near shore as possible. Bottom depths averaged approximately 120 m (range: 25–220 m). The grid was surveyed twice in summer and once, partially, each autumn. Hydroacoustic and hydrographic profile data were collected but are presented elsewhere.[3,4,5] Where fish were detected with hydroacoustic equipment, we either interrupted the survey or returned after the transect was completed to fish with a mid-water beam trawl. The net was generally fished 20–35 minutes each trawl.[3,4] The trawl's effective mouth opening was 50 m^2, and net mesh sizes diminished from 5 cm in the wings to 1 cm in the codend. A 0.3-cm mesh liner was sewn into the codend, which terminated in a plankton bucket having 500-µm nytex mesh. In summer, beach-seine and dip-net samples occasionally supplemented the trawl catches. Subsamples of forage species (n=10 to 15 per species) were preserved in 10% buffered formalin-seawater solution on the vessels for later stomach analysis in the laboratory. We classified samples collected between 08:00 and 20:00 as "day" and those between 20:01 and 07:59 as "night."

In 1995, the zooplankton prey spectrum was assessed from dual vertical hauls taken at each station within two hours of fish catches by using conical nets that were 0.5 m in diameter and equipped with 303-µm mesh in summer and 243-µm mesh in autumn. We towed the nets from a standard depth of 20 m or to the depth at which fish were caught (or using a combination of both depths). Depth of hauls were categorized as "shallow" (≤25 m) or "deep" (>25 to <100 m). Samples were collected at both depths at seven stations in summer (from 95-1-53 to 95-1-62 and 95-1-112) and one station in autumn (95-2-7, Table 1).

Laboratory methods

After a minimum of six weeks in formalin solution, fish samples were transferred to a solution of 50% isopropanol for at least 10 days before stomach analysis was performed. Ten specimens of each species were measured (mm fork length, FL; mg wet weight), and size was used to develop age-class categories for diet samples (Smith, 1981; Paul et al., 1998a). Walleye pollock (20 to 120 mm FL) and Pacific herring (60 to 120 mm FL) were classified as YOY (age-class 0). Stomachs were excised, weighed, and their contents were removed. The weight of prey contents was recorded as the difference between full and empty stomach weights. Fish were considered to have been feeding if their stomachs contained more than a trace of food. Relative stomach fullness was recorded as integers representing empty stomachs (1), stomach containing trace contents (2), stomachs that were 25%, 50%, 75%, or 100% full (3–6), or stomachs that were distended (7). State of digestion was recorded as partially digested contents (1), mostly digested contents (2), and empty stomachs (3).

Stomach contents and zooplankton samples were identified with a binocular microscope to the highest taxonomic resolution possible and enumerated. The prey category of calanoid copepods was also segregated into "large" (≥2.5 mm total length, TL) and "small" individuals (≥2.5 mm TL). We pooled the common pelagic cyclopoid copepod *Oithona similis* with small calanoids. We subsampled all zooplankton samples and stomach samples when practical, using a Folsom splitter to achieve a minimum count of 200 of the predominant taxon. Counts were expanded and total prey weights were determined by multiplying the expanded number observed by the mean weight per taxon. Weights per taxon were obtained from data on file (specimens from zooplankton samples or fish stomachs) collected from spring through autumn of various years in southeastern Alaska or PWS (Coyle et al., 1990; Stark[6]; senior author, unpubl. data).

Analytical methods

Forage fish were considered to occur in allopatric aggregations if only one species and one age class were caught in a net haul. They were considered to be sympatric if at least two species or age classes (≥10 fish each) were caught together. For this study, we restricted analyses to YOY pollock and herring that were allopatric or that co-occurred only with each other to limit the complexity of trophic interactions; we excluded pollock and herring that were caught in other types of aggregations, such as with other species or older conspecifics. We examined the size of forage fish and their feeding attributes. Size included FL and wet weight. Feeding attributes included measures of the quantity of food consumed, measures of feeding frequency, and measures of prey composition. Food quantity was expressed as means of the total number and weight of prey (ln-transformed), stomach fullness index (rounded to nearest 25%), and prey percent body weight (%BW; ratio of wet stomach-content weight to fish body weight). Feeding frequency was measured as the percentages of feeding fish and the percentages of fish with partially or mostly digested stomach contents. Prey composition was expressed as the percent number and percent biomass of prey categories. Zooplankton density per cubic meter and numerical percent composition was calculated for species,

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[3] Haldorson, L. 1995. Fish net sampling. *In* Forage fish study in Prince William Sound, Alaska, p. 55–83. *Exxon Valdez* Oil Spill Restoration Project 94163A Annual Report, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801.

[4] Haldorson, L. J., T. C. Shirley, and K. O. Coyle. 1996. Biomass and distribution of forage species in Prince William Sound. *In* APEX project: Alaska predator ecosystem experiment in Prince William Sound and the Gulf of Alaska (D. C. Duffy, compiler), Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95163 A–Q), Alaska Natural Heritage Program, Department of Biology, Univ. Alaska Anchorage, 707 A Street, Anchorage, AK 99501.

[5] Haldorson, L. T. Shirley, K. Coyle, and R. Thorne. 1997. Forage species studies in Prince William Sound. *In* Alaska predator ecosystem experiment in Prince William Sound and the Gulf of Alaska (D. C. Duffy, compiler). Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 96163 A–Q), Alaska Natural Heritage Program, Department of Biology, Univ. Alaska Anchorage, 707 A Street, Anchorage, AK 99501.

[6] Stark, C. 1995. Personal commun. Institute of Marine Science, Univ. Alaska Fairbanks, P. O. Box 757220, Fairbanks, AK 99775-7220.

Table 1

Mean zooplankton density and percent density (standard errors, SE, in parentheses) from pooled vertical hauls in summer (n=37 hauls, 303-µm mesh) and autumn (n=8 hauls, 243-µm mesh), and from shallow (≤ 25 m) and deep (50–100 m) hauls in Prince William Sound, 1995.

	Mean density (number of zooplankton/m^3)					
	Summer			Autumn		
Prey category	Overall	Shallow	Deep	Overall	Shallow	Deep
Barnacle larvae	<1	<1	<1	<1	<1	<1
Calanoids, large	29 (4)	37 (7)	28 (6)	204 (60)	104 (3)	176 (3)
Calanoids, small	1018 (133)	1175 (181)	550 (87)	828 (130)	685 (48)	426 (40)
Chaetognaths	4 (<1)	3 (1)	4 (1)	3 (<1)	1 (<1)	5 (<1)
Cladocera	33 (9)	25 (9)	6 (1)	0	0	0
Cyphonautes larvae	0	0	0	205 (50)	297 (7)	333 (8)
Decapod larvae	1 (<1)	2 (<1)	<1	0	<1	0
Euphausiid larvae	3 (1)	3 (1)	2 (<1)	2 (<1)	1 (<1)	2 (1)
Gastropods	60 (10)	99 (23)	36 (5)	96 (19)	141 (4)	84 (5)
Hyperiid amphipods	2 (<1)	2 (<1)	2 (<1)	1 (<1)	1 (<1)	1 (<1)
Larvaceans	14 (4)	6 (2)	8 (3)	45 (11)	50 (12)	22 (7)
Other	17 (2)	17 (3)	8 (1)	14 (2)	18 (6)	9 (1)
Total[1]	1184 (138)	1371 (191)	645 (91)	1414 (185)	1299 (64)	1064 (56)

	Percent composition					
	Summer			Autumn		
Prey category	Overall	Shallow	Deep	Overall	Shallow	Deep
Barnacle larvae	0	0	0	0	0	0
Calanoids, large	3	3	5	13	8	17
Calanoids, small	84	84	84	58	53	40
Chaetognaths	<1	<1	<1	<1	<1	<1
Cladocera	5	2	1	0	0	0
Cyphonautes larvae	0	0	0	16	23	14
Decapod larvae	0	0	0	0	0	0
Euphausiid larvae	<1	<1	<1	<1	<1	<1
Gastropods	6	7	6	7	11	8
Hyperiid amphipods	<1	<1	<1	<1	<1	<1
Larvaceans	<1	<1	<1	2	4	2
Other	2	1	1	1	1	1

[1] Numbers do not add to column totals because of rounding.

principal prey taxa, and total organisms in each vertical tow by using the expanded organism count divided by the water volume of the tow; mean values of replicate tows were used to represent each station.

Feeding selectivity of allopatric and sympatric aggregations of pollock and herring was calculated for summer and early autumn, 1995, when zooplankton were collected at the fish sampling stations. Occasionally, in summer, zooplankton samples from adjacent stations were substituted for those fishing stations without prey samples (Table 1). At stations where zooplankton was collected at two depths, the selection values presented were based on zooplankton from the depth where fish diet samples were collected. We used Strauss' linear selection index, L_0 (Strauss, 1979), a measure varying from –1 to +1, where negative values indicate no preference for the prey taxon and positive values indicate preference for the prey taxon:

$$L_0 = r_i - p_i,$$

where r_i = percentage of i^{th} prey resource in the diet; and
p_i = percentage of i^{th} prey resource in the environment.

Prey resources for selection were defined as the species, stages, and sizes of prey pooled into principal taxa.

Feeding overlap between species and within species between fish in allopatric and sympatric aggregations was described by using Horn's overlap index (Horn, 1966; Smith and Zaret, 1982; Krebs, 1989). This index minimizes bias due to changing numbers of resource categories and resource evenness. Overlap was computed at two levels: prey resources were defined at the lowest level (species, stage, and size) and at a pooled level (principal taxa). Horn's overlap index values, R_0, were expressed from 0 (no overlap) to 1 (total overlap) for predator species j and k:

$$R_0 = \frac{\sum (p_{ij} + p_{ik}) \times \ln(p_{ij} + p_{ik}) - \sum p_{ij} \times \ln p_{ij} - \sum p_{ik} \times \ln p_{ik}}{2 \times \ln 2}$$

where p_{ij} = proportion of i^{th} resource in total prey resources utilized by j^{th} species; and
p_{ik} = proportion of i^{th} resource in total prey resources utilized by k^{th} species.

We considered R_0 values >0.60 to indicate similar use of resources and R_0 >0.75 to indicate very similar use of resources.

In this report, we examined both intraspecific and interspecific differences in YOY pollock and herring and qualitatively compared data between seasons, between years (in autumn), between allopatric and sympatric aggregation types, and between sympatric species. We also compared day-night feeding frequencies and prey condition to assess principal time of day of feeding. We compared zooplankton densities and percent densities of principal taxa between seasons and between depths (shallow and deep) sampled each season. Because our data were limited and the sampling design unbalanced, we present means and standard errors without statistical tests.

Results

Zooplankton prey fields

Total zooplankton densities for all samples pooled were similar in summer and autumn of 1995, averaging approximately 1200 and 1400 organisms/m^3 (n=37 and 8, mesh=303 µm and 243 µm, respectively; Table 1, Fig. 2). Approximately 3/4 of samples from each season were collected in daylight, between 10:00 and 20:00.

Zooplankton taxa were less diverse in summer than in autumn, but small calanoids predominated in both seasons. Seasonal differences in the density and percentage contribution of a few taxa were apparent. Small calanoids made up a greater percentage of the total in summer compared with autumn (84% vs. 58%), but their density was similar in each season. Large calanoid density and percent density were both lower in summer than in autumn. Only a few other taxa contributed ≥5% to the total numbers of zooplankters in either season. In summer, these included cladocera and gastropods (primarily the pteropod *Limacina helicina*); in autumn, they included large calanoids, bryozoan cyphonautes larvae, and gastropods (Table 1, Fig. 2).

Calanoid species composition in the zooplankton also varied seasonally. Small calanoids in summer were predominantly *Pseudocalanus* spp. (75%) and *Acartia longiremis* (5%); in autumn, *Pseudocalanus* spp. was 40%, *Oithona similis,* 15%, and *A. longiremis,* 6% of total zooplankton. Large calanoids in summer were principally *Neocalanus* and *Calanus* spp. (3% of total zooplankton) and in autumn, they were *Metridia pacifica* (10%) and *Calanus* spp. (1%). Among other taxa present in both seasons, density and percent density of gastropods were similar between seasons, but larvaceans were less available in summer than in autumn. Cladocera were present only in summer and cyphonautes only in autumn. Hyperiid amphipods, euphausiid larvae, chaetognaths, and barnacle and decapod larvae were rare in both seasons (Table 1).

Zooplankton density per cubic meter varied with depth of sampling at all stations for both seasons. In summer (n=7 stations), zooplankton density was more than twice as high in shallow hauls as in deep hauls, whereas in autumn (n=1 station), it was greater in the shallow tows than in the deep tows (Table 1). Depth-related differences in the abundance of principal taxa, but not in their percentage composition, were also observed. Overall, in summer, small calanoids were twice as abundant in the more shallow tows, but they comprised similar percentages at each depth. In autumn, both density and percentage of small calanoids were greater in shallow tows than in deeper tows. Large calanoid density and percentage did not differ between depths in summer, but in autumn they were lower in shallow tows compared with deep tows. Gastropod abundance in both seasons, and cladoceran abundance in summer, were greater in shallow tows than in deep tows, but percentages did not differ with depth. Larvacean abundance and percentage abundance in either season, and the abundance and percentage abundance of cyphonautes in autumn, did not differ between depths.

The macrozooplankters, larval euphausiids and young hyperiids, were present, but rare in our plankton tows. However, euphausiids were captured in 11% of summer trawls and in 43% of autumn trawls. In summer of 1995,[4] juvenile–adult euphausiids were present in the northeastern and southwestern regions of the sound but were not caught in the central region or at any stations where fish were caught; in autumn (1994[7] and 1995[4]), they were present in trawls from all three regions but were absent from the allopatric trawl in October 1995. We have no consistent data on the presence or absence of hyperiid amphipods.

Fish catches

Catches of YOY walleye pollock and Pacific herring sampled from PWS that met our allopatric-sympatric criteria (see "Materials and methods" section) were not evenly rep-

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[7] Paul, A. J. 1995. Invertebrate forage species. *In* Forage fish study in Prince William Sound, Alaska, p. 43–54. *Exxon Valdez* Oil Spill Restoration Project 94163A Annual Report, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801.

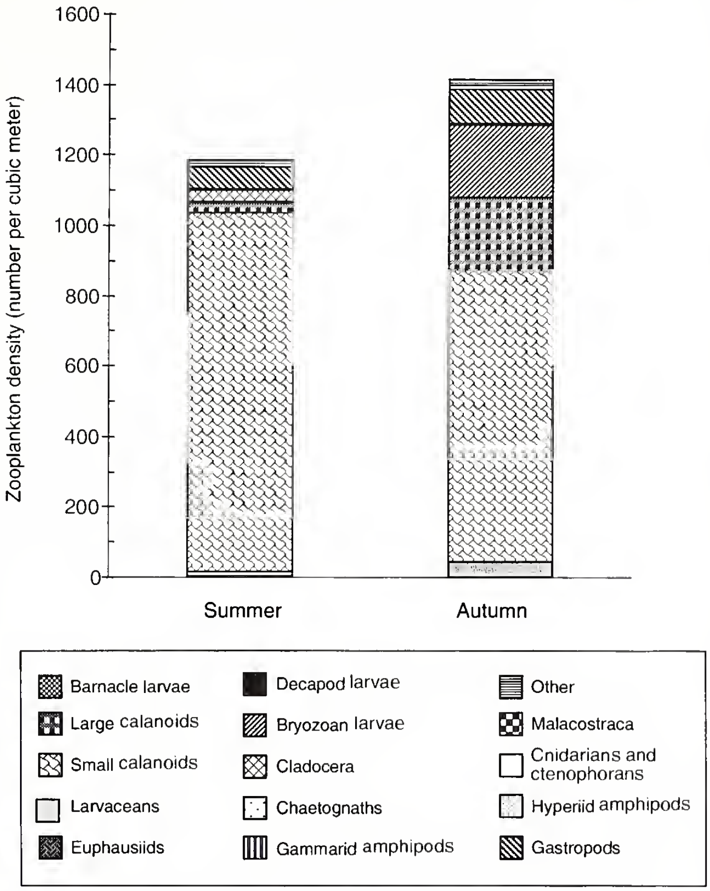

Figure 2

Seasonal zooplankton density and composition by principal taxa in Prince William Sound, Alaska 1995. Zooplankton were collected in vertical tows of conical nets having 303-µm mesh in summer and 243-mm mesh in autumn.

resented in both seasons. Only allopatric fish qualified in the summer of 1995, but both allopatric and sympatric fish qualified in the autumns of 1994 and 1995 (Table 2). In summer, 18 of the 62 trawl hauls caught sufficient samples of either species.[4] Allopatric YOY pollock were collected at 12 stations in the central region and allopatric YOY herring were collected at one central and one northeastern station in summer (Fig. 1). In autumn, 11 of the 25 trawl hauls (total 14 in November 1994,[3] 11 in October 1995[4]) caught sufficient samples of either species, and 36% of these caught sympatric fish in the northeastern region. Allopatric species were collected in different years: the allopatric pollock in November 1994 were collected in the southwestern region and the allopatric herring in October 1995 in the central region (Table 2). The remainder of pollock and herring caught did not meet our study criteria for age, number, or composition of fish per haul.

The magnitude and relative species composition of the catch varied considerably, and sampling time and depth differed between seasons. In summer, the number of pollock caught in trawls varied by two orders of magnitude between stations, from 22 to 1689 per haul (Table 2). Herring catches in the alternative gear, dip net or beach seine, were of similar magnitude. In autumn, from 14 to 4156 pollock or herring were caught per trawl haul; the catches were not evenly partitioned between the species in sympatric hauls. Similar numbers of pollock and herring were caught at some sympatric stations, whereas, at others, the number per species caught was an order of magnitude greater. In summer, approximately half the samples were collected in the morning, half in the afternoon, and only one was collected at dusk (~22:00). The pollock were caught most often at 50–80 m trawl depths ($\bar{x}$=60 m) offshore and the herring were caught at the surface and near shore. In autumn, sympatric samples were collected in darkness (approximately 22:00–23:00) at a mean depth of 30 m in bays, whereas allopatric samples were collected during daylight and in deeper water (Table 2).

Table 2

Sampling stations, catch, mean size, and feeding attributes for allopatric and sympatric YOY walleye pollock and Pacific herring (n=10 each) from locations in PWS in summer 1995 and autumns 1994–1995. Zooplankton samples were collected at most fishing stations only in 1995; asterisks indicate stations where an adjacent zooplankton haul was substituted. Standard error of the means (SE) is shown in parentheses. Abbreviations: T = trawl, D = dip net, B = beach seine, NE = northeast, C = central, SW = southwest.

Sampling period, target catch, cruise number, and gear	Region and location	Number caught	FL (mm)	% feeders	Fullness (%)	Content %BW	Total number of prey	Total weight of prey (mg)
Summer 1995								
Allopatric Pacific herring								
95-1-27D*	NE, Fish Bay, Port Fidalgo	271	76 (2.0)	100	100	3.8 (0.4)	5968.7 (604.3)	540.71 (62.8)
95-1-110B*	C, Southeast Eleanor Is.	—[1]	30 (0.3)	60	25	0.0 (0.0)	52.6 (28.6)	1.55 (0.7)
Allopatric walleye pollock								
95-1-3T	C, Applegate Rocks	74	59 (1.5)	60	25	0.7 (0.2)	171.0 (113.0)	14.25 (7.5)
95-1-5T	C, Seal Island	31	54 (1.2)	60	50	1.2 (0.3)	37.1 (14.1)	27.27 (12.0)
95-1-19T*	C, Lilhengren Passage	139	58 (1.7)	80	50	1.8 (0.5)	12.4 (3.6)	12.11 (5.0)
95-1-53-1T	C, Montague Point	—[1]	56 (1.1)	100	75	1.1 (0.1)	154.1 (27.5)	13.35 (2.4)
95-1-53-2T	C, Montague Point	694	62 (1.3)	100	75	1.2 (0.2)	261.5 (38.9)	34.84 (5.7)
95-1-54T	C, East Knight Island	1056	54 (1.6)	60	25	1.3 (0.3)	53.5 (30.2)	4.68 (2.2)
95-1-56T	C, Seal Island	1689	58 (1.8)	50	25	0.7 (0.2)	21.8 (10.5)	4.86 (2.1)
95-1-57T	C, Seal Island	386	58 (2.6)	80	50	1.4 (0.5)	45.0 (19.6)	38.59 (26.0)
95-1-58T	C, Knight-Seal Island	843	56 (2.1)	100	100	2.2 (0.4)	242.0 (84.8)	33.70 (9.6)
95-1-62-2T	C, East Eleanor Island	293	53 (1.1)	90	50	1.4 (0.2)	17.2 (4.3)	15.99 (7.8)
95-1-108T*	C, Southeast Knight Is.	22	66 (1.4)	100	100	2.7 (0.4)	2489.5 (351.1)	193.24 (24.6)
95-1-112T	C, South Naked Island	504	65 (2.8)	90	50	0.9 (0.1)	20.6 (4.5)	14.79 (5.0)
October 1995								
Allopatric Pacific herring								
95-2-5T	C, East Naked Island	89	92 (1.4)	100	50	1.4 (0.1)	385.9 (89.5)	103.36 (19.5)
Sympatric Pacific herring								
95-2-6T	NE, Galena Bay	425	84 (4.3)	90	75	1.2 (0.2)	927.1 (303.6)	58.63 (11.4)
95-2-7T	NE, Landlocked Bay	592	95 (3.3)	90	50	0.5 (0.1)	271.1 (103.7)	84.50 (62.3)
Sympatric walleye pollock								
95-2-6T	NE, Galena Bay	4156	93 (2.8)	100	100	1.2 (0.2)	78.3 (14.2)	61.26 (14.4)
95-2-7T	NE, Landlocked Bay	535	90 (2.4)	90	50	0.5 (0.1)	46.8 (15.0)	16.79 (3.9)
November 1994								
Allopatric walleye pollock								
94-2-5T	SW, Icy Bay	61	111 (1.6)	100	100	1.8 (0.4)	722.6 (156.2)	71.62 (11.4)
Sympatric Pacific herring								
94-2-6T	NE, Galena Bay	26	93 (4.1)	20	10	0.1 (0.0)	13.8 (9.0)	0.78 (0.5)
94-2-7T	NE, Port Gravina	398	97 (4.2)	70	25	0.5 (0.2)	31.8 (14.7)	25.64 (12.7)
Sympatric walleye pollock								
94-2-6T	NE, Galena Bay	145	100 (3.1)	70	50	0.5 (0.5)	18.5 (5.1)	18.29 (9.2)
94-2-7T	NE, Port Gravina	14	111 (1.7)	60	25	0.4 (0.1)	6.4 (1.9)	14.56 (7.5)

[1] Data missing.

Table 3

Seasonal fish size and feeding attributes of allopatric and sympatric YOY walleye pollock and Pacific herring from Prince William Sound, 1994–1995. Values are mean and standard error, SE, for fish pooled from stations shown in Table 1.

Species and sampling period	*n*	FL	Wet weight (g)	% non-feeders	Fullness (%)	Content % BW	Total number of prey	Total weight of prey (mg)
Walleye pollock								
Summer (allopatric)	120	58.0 (1.0)	1.1 (0.1)	19	50 (5)	1.4 (0.1)	294 (68)	34.0 (5.6)
Oct. 1995 (sympatric)	20	91.5 (2.4)	5.0 (0.3)	5	75 (10)	0.8 (0.3)	63 (11)	39.0 (8.9)
Nov. 1994	30	107.2 (2.0)	8.1 (0.3)	23	50 (10)	0.9 (0.2)	249 (80)	34.8 (7.2)
Allopatric	10	111.0 (1.6)	9.1 (0.4)	0	100 (5)	1.8 (0.4)	723 (156)	71.6 (11.4)
Sympatric	20	105.4 (2.1)	7.6 (0.5)	35	25 (10)	0.4 (0.1)	13 (3)	16.4 (5.8)
Pacific herring								
Summer (allopatric)	20	52.7 (2.4)	1.5 (0.3)	20	75 (10)	1.9 (0.5)	3011 (739)	271.1 (69.0)
Oct. 1995	30	90.2 (2.0)	6.6 (0.3)	7	50 (5)	1.0 (0.1)	528 (119)	82.2 (21.6)
Allopatric	10	91.6 (1.4)	6.8 (0.4)	0	50 (10)	1.4 (0.1)	386 (90)	103.4 (19.5)
Sympatric	20	89.5 (3.0)	6.5 (0.6)	10	75 (10)	0.9 (0.1)	599 (173)	71.6 (31.0)
Nov. 1994 (sympatric)	20	94.6 (2.4)	6.9 (0.3)	55	10 (10)	0.3 (0.1)	23 (9)	13.2 (6.8)

Size of YOY pollock and herring

Intraspecific patterns of size across seasons differed for YOY pollock and herring. Mean FLs for both species were approximately 60% longer in autumn than in summer. Herring size was similar in autumn of both years, but pollock were 14 mm longer in FL and 50% heavier in November 1994 than in October 1995 (Tables 2 and 3). Interspecific comparisons of size showed that YOY pollock were generally longer, but not heavier, than YOY herring in the same season.

Feeding habits of YOY pollock and herring

Prey composition of YOY pollock was similar to that of YOY herring in both summer and autumn (Figs. 3 and 4), but prey compositions differed between seasons. Small prey predominated in summer and larger prey in autumn, especially in terms of biomass composition.

In summer, small calanoid copepods (*Pseudocalanus* spp., *Centropages abdominalis*, and *Acartia longiremis*) dominated the diets, both numerically and in terms of prey biomass (Figs. 3 and 4). For pollock, small calanoids constituted 55% by number and 57% by weight of diet. By number, most of the remainder of summer pollock diet comprised minute invertebrate eggs (39%); by weight, the remainder was large calanoids (principally *Calanus pacificus*, *C. marshallae*, and *Metridia pacifica*), fish, hyperiid amphipods, and euphausiids (both larvae and older stages, including *Thysannoessa* sp.). Pollock also commonly consumed small amounts of other prey, such as larvaceans, gastropods, and chaetognaths. For herring, small calanoids represented proportionally more of the diet than for pollock in summer. Small calanoids made up 77% by number and 88% by weight of herring diet; they were the sole taxon consumed by the YOY herring at Eleanor Island station 110 (Table 2). Most of the rest of herring diet comprised other small prey (cladocerans, bivalve larvae, and minute invertebrate eggs), whereas decapod larvae, gastropods, hyperiids, and euphausiid larvae made minor contributions.

In autumn of both years, pollock and herring again fed on the same prey categories. Compared with summer, small calanoids composed smaller percentages of the diet (only up to 37% of prey number and 9% of prey biomass). Larvaceans and large calanoid copepods numerically dominated their diets (57–91%; Fig. 3), whereas euphausiids and large calanoids generally dominated in terms of biomass percentages (49–89%; Fig. 4). Hyperiids occasionally contributed substantial dietary biomass. Fish in all of the autumn aggregations ate a variety of sizes of juvenile–adult euphausiids (*T. raschii, T. spinifera,* and unidentifiable species), and amphipods (*Themisto pacifica*, *Primno macropa*, and *Hyperia* sp.), but not the larval stages observed in summer diets. The large calanoids consumed included the same species present in summer diets, as well as *M. okhotensis* and *Neocalanus* spp.; the small calanoids included *Pseudocalanus* spp., *Acartia longiremis*, and *Oithona similis*. Some differences in autumn diet composition existed between years. Invertebrate eggs (the majority of "other") were present less frequently in autumn diets than in summer diets. In general, the October 1995 diets included proportionally more biomass from large calanoids, whereas the November 1994 diets included proportionally more biomass from euphausiids and proportionally more numbers from larvaceans.

Diet overlap between YOY pollock and herring was high, particularly when prey species were grouped into principal taxa (Table 4). In summer, diets were very similar ($R_0 \geq 0.76$) between allopatric species in terms of numbers and weights of prey species or principal prey taxa. In autumn, interspecific diet overlap was also observed

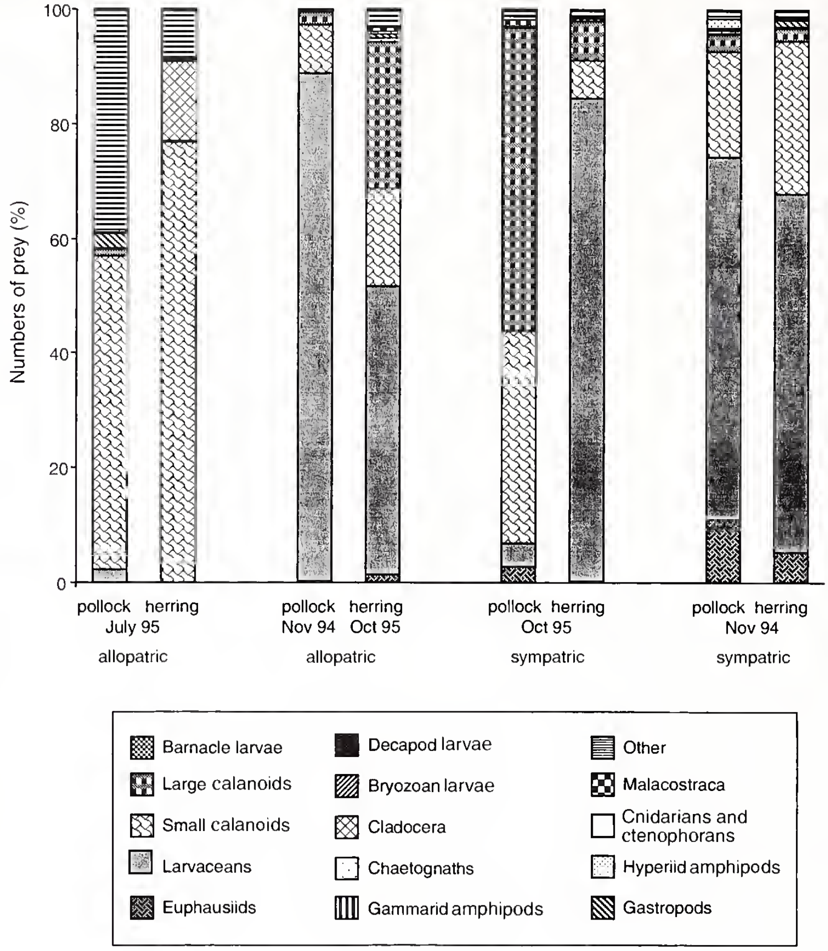

Figure 3

Percent total number of prey consumed by YOY walleye pollock and Pacific herring from sympatric and allopatric aggregations in Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994.

in terms of prey numbers and weights. Numeric overlap for the November 1994 sympatric species was observed at both stations, and the mean was approximately twice that for the October 1995 sympatric species (R_0=0.97 versus 0.43). Biomass overlap between sympatric fish occurred at three out of four autumn stations when based on principal prey taxa, but only at one station (in November) when based on prey species. Overall, diets of sympatric pollock and herring overlapped more in terms of biomass in November (R_0=0.95) than in October (R_0=0.69). We did not have autumn samples from the same year to compare allopatric diet overlap between species.

Prey selection

We noted selection from zooplankton prey resources by pollock in summer and by pollock and herring in autumn, 1995 (Fig. 5). Selection patterns were almost identical whether calculated from shallow or deep zooplankton abundances; values were always in the same direction (selection or avoidance), and their magnitude was within 10 points. Neither species selected small calanoids, the most abundant zooplankton taxon in both seasons. In summer, even though small calanoids made up >50% of YOY pollock and herring diets (Figs. 3 and 4), this taxon was avoided by pollock and was consumed in close proportion to its availability by herring (Fig. 5). Summer pollock moderately selected for large calanoids, gastropods, and larvaceans, but summer herring did not strongly select for any prey category.

Changes in zooplankton composition from summer to autumn were reflected in fish diets and prey selection. The percent density of small calanoids in the zooplankton declined by nearly 30% from summer to autumn, the percentage consumed by fish was likewise much reduced, and selection values were more strongly negative. In contrast, both large calanoids and larvaceans were more abundant in zooplankton samples in autumn than in summer and,

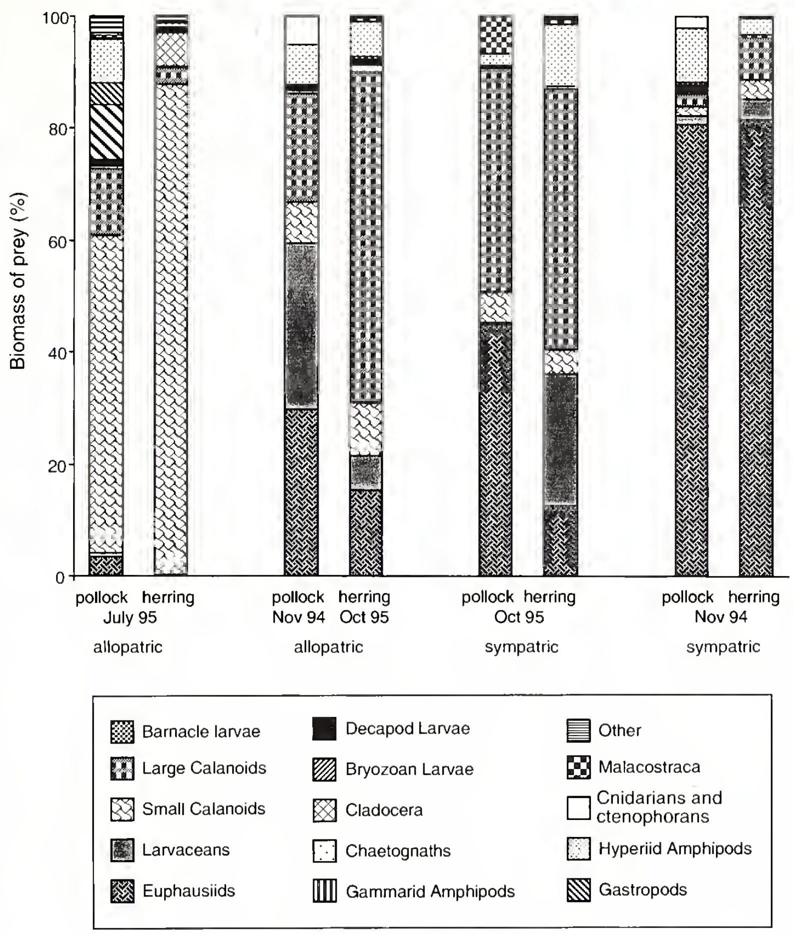

Figure 4

Percent total biomass of prey consumed by YOY walleye pollock and Pacific herring from sympatric and allopatric aggregations in Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994.

along with euphausiids and hyperiids, formed larger dietary components at that time. In autumn (October 1995), pollock strongly selected for large calanoid copepods and herring strongly selected for larvaceans (Figs. 3–5). Additional differences were noted in terms of prey frequency. In summer, pollock consumed hyperiids more frequently than they consumed euphausiids, and in autumn, both pollock and herring consumed euphausiids more frequently than hyperiids.

Seasonal feeding

Several measures of feeding in autumn indicated that YOY pollock continued to feed moderately, whereas the pattern for YOY herring showed more of a decline from summer (Tables 1 and 3). Very similar high percentages of pollock and herring had fed in summer and in October 1995 (≥80%), but in November 1994, the percentage of nonfeeding herring was greater than the percentage of feeding herring, and was more than twice the percentage of nonfeeding pollock (Table 3). For pollock, the November 1994 feeding measures were only lower than the high summer measures, whereas for herring, feeding measures in autumn of 1994 were lower than in either summer or autumn of 1995. Although pollock stomachs were at least half full in each season, herring stomach fullness declined from 75% full in summer to half full in October 1995, to only trace amounts of food in November 1994. Similarly, prey content %BW declined less between seasons for pollock than for herring. Total prey numbers and weights were generally lower for pollock than for herring in a season, but numbers of prey were highly variable and biomass of prey was relatively stable for pollock, whereas these measures showed declining trends for herring (Fig. 6, Table 3).

Digestion data and feeding-frequency data for individual fish were pooled across seasons to compare day and night feeding patterns. The condition of stomach contents

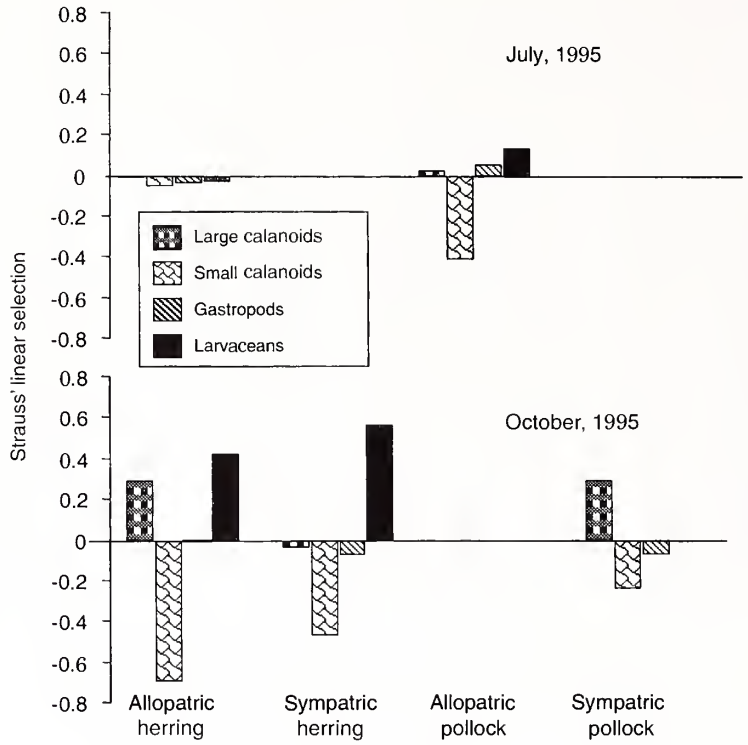

Figure 5

Strauss' selectivity index (mean) for principal prey groups of allopatric and sympatric YOY walleye pollock and Pacific herring from PWS in July–August and October 1995. Selection was not computed for November 1994, when zooplankton were not collected. Positive values indicate selection, negative values avoidance.

Table 4

Horn's overlap index values for total numbers and biomass of prey consumed by YOY walleye pollock and Pacific herring caught separately in summer and together in autumn in Prince William Sound, 1994–1995. No summer sympatric fish were available and autumn allopatric fish were not caught in the same year. Overlap greater than 0.60 indicates similar diets (see text). C = central; NE = northeast.

			Overlap in number		Overlap in biomass	
Sampling period and target catch		By prey Region	By prey species	By prey category	By prey species	category
Interspecific diet overlap						
July 1995 allopatric fish	1995	C, NE	0.79	0.82	0.76	0.83
October 1995 sympatric fish	1995–96	NE	0.16	0.22	0.44	0.64
	1995–97	NE	0.43	0.48	0.53	0.69
	Average	NE	0.31	0.43	0.55	0.69
Intraspecific diet overlap						
November 1994 sympatric fish	1994–96	NE	0.69	0.94	0.08	0.39
	1994–97	NE	0.86	0.91	0.88	0.91
	Average	NE	0.87	0.97	0.88	0.95
Allopatric-sympatric fish						
October 1995 herring			0.51	0.89	0.56	0.93
November 1994 pollock			0.87	0.91	0.56	0.73

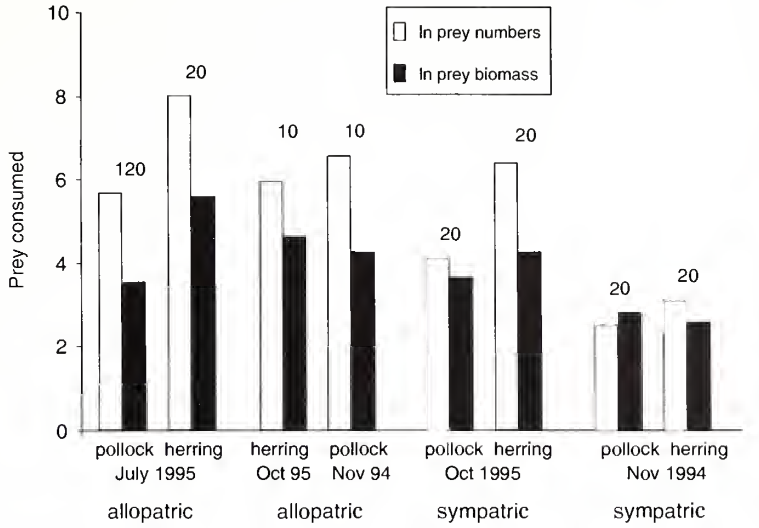

Figure 6

Total number and total biomass of prey (ln-transformed means) consumed by allopatric and sympatric YOY walleye pollock and YOY Pacific herring from Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994. Number of individuals is indicated.

by time of day was different for the two species. Pollock had greater percentages of mostly digested contents during the day than during the night (before and after 22:00). Conversely, herring had greater percentages of mostly digested contents during the night than during the day. The day-night percentages of feeding and nonfeeding fish were similar for both, however.

Comparisons between allopatric and sympatric fish aggregations

The allopatric-sympatric size pattern was different for pollock and herring. We did not pool sympatric fish from October 1995 and November 1994 for comparison with allopatric groups because of the interannual differences in size and feeding measures. For pollock in November 1994, FLs of allopatric and sympatric fish were similar, but fish were approximately 1.5 g lighter in sympatric aggregations than fish in allopatric aggregations. For herring in October 1995, the sizes of allopatric and sympatric fish were similar (Table 3).

The allopatric-sympatric feeding pattern was somewhat different for pollock than for herring in autumn. Among November 1994 pollock, the allopatric fish consistently had the highest feeding measures and the sympatric fish consistently had the lowest feeding measures (Fig. 6, Table 3). This finding coincided with slightly increased numerical percentages of larvaceans, increased gravimetric percentages of euphausiids, and increased frequencies of euphausiids, amphipods, and large calanoids in the diet of allopatric pollock compared with the diet of sympatric pollock (Figs. 3 and 4). For October 1995 herring, allopatric fish consumed the greatest prey biomass and %BW, but their prey numbers and fullness index were lower than those of sympatric fish (Fig. 6, Table 3). This finding coincided with decreased numerical percentages of larvaceans, increased numerical and gravimetric percentages of large calanoids and euphausiids, and increased frequencies of occurrence of hyperiids and euphausiids in the diet of allopatric herring compared with diet of sympatric herring (Figs. 3 and 4). Allopatric herring were more selective of large calanoids, but selection for larvaceans was similar for both groups of herring (Fig. 5). For both species, intraspecific diet overlap between allopatric and sympatric fish was extensive at the principal taxon level. In October 1995 intraspecific overlap in terms of prey biomass was 0.93 for herring, and in November 1994, intraspecific overlap in terms of prey number was 0.91 for pollock.

Discussion

Zooplankton prey fields

The prey fields available to planktivorous YOY walleye pollock and Pacific herring were different in summer and autumn 1995. Although we report similar total zooplankton densities for the two seasons, we believe that summer densities of small calanoids were underestimated with the 303-µm net. This conclusion is based on a 1995 companion study that showed that a 243-µm mesh net retained small calanoids better than a 303-µm mesh net.[8] The sea-

(continued on next page)

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[8] Sturdevant, M. V. In review.

sonal densities presented in our study were not directly comparable because of the different gear used, and our summer mesozooplankton densities were lower than those reported from other summer collections in PWS[9] (Cooney et al., 1981; Celewycz and Wertheimer, 1996). The food supply available to juvenile fish was likely more abundant in summer than in autumn, because other studies in the northeastern Pacific showed a steady decline in zooplankton biomass from summer to winter (Peterson and Miller, 1977; Cooney, 1986; Incze et al., 1997; Foy and Paul, 1999). Zooplankton taxonomic compositions also differed seasonally, mainly in the larger percentages of alternate prey available in autumn.

We found the surface-water feeding environment to be richer in numbers of prey than the deeper water in both seasons. Lower zooplankton density with depth has been reported by other authors in PWS,[10] Shelikof Strait (Napp et al., 1996), off the Oregon Coast (Marlowe and Miller, 1975), and at Ocean Station "P" (Petersen and Miller, 1977). Large calanoids were an exception in autumn, however, when their densities and percentages were greater in the deeper tows, typical of subarctic locations (e.g. Marlowe and Miller, 1975; Nakatani, 1988; Napp et al., 1996).

High abundances of *Pseudocalanus* spp. and other small calanoids and high biomass of large calanoids are characteristic of neritic locations in subarctic Pacific waters, such as those of PWS in summer (Springer et al., 1989; Coyle et al., 1990; Celewycz and Wertheimer, 1996; Incze et al., 1997). The time of collection for zooplankton is important, however, because the location of peak abundance in the water column varies with their diel vertical migration. Calanoid copepods are usually most abundant at the surface at night, migrating deeper during the day (e.g. Sekiguchi, 1975). Even though most of our samples were collected during the day, it is not surprising that abundances were greater in shallow tows than in deeper tows because *Pseudocalanus* was dominant. Of the species important in our study, *Pseudocalanus newmani* was typically the shallowest in depth distribution, *Calanus pacificus* was intermediate and *Metridia pacifica*, the deepest (e.g. Bollens and Frost, 1989; Frost and Bollens, 1992; Bollens et al., 1993; Bollens et al., 1992b). However, the vertical distribution patterns of these species were influenced by the presence of predators and other factors (e.g. Bollens and Frost, 1989; Bollens et al., 1992b; Frost and Bollens, 1992; Bollens et al., 1993). The seasonal difference in depth distribution of large calanoids could have been due to seasonal vertical migrations (Sekiguchi, 1975; Mackas et al, 1993), differences in sampling time, or a response to the changing light regime in autumn.

Feeding habits

Summer and autumn diets of YOY allopatric and sympatric walleye pollock and Pacific herring in PWS were similar to those reported from other areas and to those of other pollock and herring caught during the study that did not meet our criteria (Sturdevant, unpubl. data; see "Materials and methods" section). In summer, both species consumed the abundant calanoid taxa as well as less abundant small prey. Calanoid copepods were likewise the predominant summer prey of YOY pollock in Japanese waters (Kamba, 1977; Nakatani, 1988), the region of the Kodiak Island-Alaska Peninsula,[11] the Gulf of Alaska and eastern Bering Sea (Grover, 1990, 1991; Brodeur et al., 1997), PWS (Willette et al., 1997; Foy and Norcross, 1999a), and southeastern Alaska.[12] The small calanoid *Acartia clausi* was particularly important in southeastern Alaska diets from August to October,[12] but by late summer in other areas, euphausiids accounted for more prey biomass and calanoids continued to dominate numerically (Merati and Brodeur, 1996). The autumn prey composition of pollock in our study was also similar to that of YOY pollock in the Gulf of Alaska (Merati and Brodeur, 1996; Brodeur et al., 2000), eastern Kamchatka (Sobolevskii and Senchenko, 1996), and southeastern Alaska.[12] In those studies, increased fish size was correlated with decreased predation on small copepods and increased predation on large copepods, larvaceans, and euphausiids. By winter, large copepods virtually disappeared from diets in some areas (Sobolevskii and Senchenko, 1996); chaetognaths and epibenthic prey such as mysids, shrimps, caprellid amphipods, and cumaceans were incorporated in the diet as vertical distributions of the fish changed and pelagic prey became scarce[12] (Merati and Brodeur, 1996; Sobolevskii and Senchenko, 1996; Brodeur et al., 2000). Seasonal changes in prey have also been correlated with change in YOY pollock distribution and the use of different habitats (Nakatani, 1988).

Like pollock, YOY Pacific herring depended on small calanoid prey in PWS and throughout their range. Additional small prey taxa are commonly reported in Pacific herring and other species diets, including invertebrate eggs, barnacle larvae, cladocerans, oikopleurans, and juvenile amphipods and euphausiids[9] (Wailes, 1936; Sherman and Perkins, 1971; Last, 1989; Arrhenius and Hans-

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[8] (*continued*) Summer zooplankton density and composition estimates from 20-m vertical hauls using three net meshes. Alaska Fisheries Research Bull., Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801.

[9] Sturdevant, M. V., and L. B. Hulbert. 1999. Diet overlap, prey selection, and potential food competition among allopatric and sympatric forage fish species in Prince William Sound, 1996. *In* Forage fish diet overlap, 1994–1996, p. 72–100. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 97163C), Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, Alaska 99801.

[10] Foy, R. J., and B. L. Norcross. In prep. Nearshore zooplankton community ecology in Prince William Sound, Alaska. J. Plankton Res., Institute of Marine Science, Univ. Alaska Fairbanks, P. O. Box 757220, Fairbanks, AK 99775-7220.

[11] Livingston, P. A. 1985. Summer food habits of young-of-the-year walleye pollock, *Theragra chalcogramma*, in the Kodiak area of the Gulf of Alaska during 1985. Unpubl. manuscr., Alaska Fish. Sci. Center, National Marine Fisheries Center, 7600 Sand Point Way NE, Seattle, WA 98115, 8 p.

[12] Krieger, K. J. 1985. Food habits and distribution of first-year walleye pollock, *Theragra chalcogramma* (Pallas), in Auke Bay, Southeastern Alaska. Unpubl. MS thesis, Univ. Alaska Juneau, 11305 Glacier Hwy., Juneau, AK 99801, 57 p.

son, 1994; Arrhenius, 1996; Haegele, 1997; Willette et al., 1997). In addition to pelagic prey, epibenthic prey such as harpacticoid copepods and gammarid amphipods were important in estuarine habitats of the Fraser River for Pacific herring and coastal Maine for Atlantic herring (Sherman and Perkins, 1971; Blaxter and Hunter, 1982; Levings, 1983, in Lassuy, 1989). Spatial differences were found for diets of Pacific herring from four widespread bays in PWS (Foy and Norcross, 1999a). We found seasonal differences in prey similar to reports by others: euphausiids replaced calanoids in the diets of older juvenile herring compared with younger juvenile herring (Wailes,1936; Lassuy, 1989; Haegele, 1997), and larvaceans (Foy and Norcross, 1999a, 1999b), mysids, and other malacostracans (Foy and Norcross, 1999a; Foy and Paul, 1999) were also more common in autumn. In Auke Bay, Alaska, juvenile herring diets varied with abundance of zooplankton prey taxa in spring and early summer and included large calanoids and euphausiids when they were present (Coyle and Paul, 1992). The marine distribution of the alewife, *Alosa pseudoharengus,* another herring, was correlated with the seasonal distribution, availability, and abundance of its euphausiid prey (Stone and Jessop, 1994).

Prey selection, feeding time, and depth

Segregation by depth is one way to reduce interspecific competition among fish with overlapping distributions (e.g. Jessop, 1990; Arrhenius, 1996). Both pollock and herring perform diel vertical migrations (Smith, 1981; Blaxter and Hunter, 1982), the pattern of which can vary seasonally and ontogenetically (e.g., Kamba, 1977; Lassuy, 1989; Olla et al., 1996; Stokesbury et al., 2000). We observed some interspecific differences in prey selection that may relate to diel vertical distributions of predator and prey, prey preferences, ontogenetic changes in prey size, or different feeding rhythms for both species. Neither species selected small calanoids in summer, and pollock selected taxa that herring did not (large calanoids, pteropods, and larvaceans). The summer herring were located nearshore and at the surface where densities of their main prey were twice as high as deep in the water column and where light for feeding was most intense. In contrast, summer pollock were located in relatively deep water with less light and lower prey densities. Even though small calanoids were the predominant prey of pollock and the predominant zooplankter, they were avoided in relation to their availability. In autumn, both species avoided small calanoids even more strongly than in summer. In autumn, sympatric pollock in shallow water selected large calanoids, mainly *Metrida pacifica*, that were less available than in deeper water, but only the deeper allopatric herring selected them. Young Pacific herring in another study selected *Calanus pacificus* over *Metridia lucens*, perhaps in relation to fine-scale differences in prey depth distributions (Fortier and Leggett, 1983 in Munk et al., 1989; Bollens et al., 1993). Both sympatric herring and allopatric herring strongly selected the larvaceans that were more evenly distributed in the water column. We primarily sampled fish schools that were located acoustically and assumed that the fish would be located where food was available (e.g. Arrhenius and Hansson, 1999). Comparisons of prey selection between seasons are valid because the calculations are based on prey percentages which did not differ between the 243- and 303-µm mesh sizes used.[8]

The less-digested condition of herring stomach contents by day compared with night suggests that the summer fish and autumn allopatric fish were actively feeding and that the autumn sympatric fish were not. The condition of pollock prey and the fact that the fish were not located where their selected prey were in either season suggest that pollock were not actively feeding at the times we sampled in either season. The change in digested state of their prey with time of day also suggests that YOY pollock may seasonally or ontogenetically switch from feeding principally during the day to feeding at night[12] (Merati and Brodeur, 1996; Brodeur et al., 2000).

Past diel studies have reported different patterns of feeding for pollock and various herring species. For example, peak time of feeding for pollock was at midnight or just before dawn in some studies (Brodeur and Wilson,1996a; Merati and Brodeur, 1996; Willette et al., 1997), but another study showed a change in feeding chronology from sunset in small fish to night in larger fish (Brodeur et al., 2000). Peak time of feeding for Atlantic, Baltic, and Pacific herring occurred in the afternoon or evening, with the lowest feeding rates in early morning (e.g. de Silva, 1973; Blaxter and Hunter, 1982; Mehner and Heerkloss, 1994; Willette et al., 1997; Arrhenius, 1998). Changes in prey composition with time of day and ontogeny have also been noted in some studies (e.g. Nakatani, 1988; Grover, 1991; Munk, 1992; Stone and Jessop, 1994) and not others (Brodeur et al., 2000). If co-occurring fish feed at different times, their diets could be highly similar without direct competition because predation on the same prey resources would be temporally or spatially separated. This could be the case with small calanoid prey, which both species fed on in summer, but only herring were co-located at the depth of prey concentration. Different feeding periodicities could result in indirect competition if prey resources are limited, however.

Sampling time could also affect the appearance in the diet of vertically migrating macrozooplankton, such as juvenile-adult euphausiids and hyperiid amphipods, because the vertical locations of peak abundance of predator and prey may not overlap continually. For example, euphausiids could be consumed at night near the surface or during the day near the bottom[12] (Pearcy et al., 1979; Nakatani, 1988). Juvenile euphausiids were rare summer prey in our study and were present only in those trawls taken below the 60-m mean depth of pollock catches.[2] In autumn, the euphausiid and fish distributions were more likely to overlap during the night sampling time (e.g. Bailey, 1989; Bollens et al., 1992a), and euphausiids were indeed a principal prey in terms of biomass, particularly for pollock. Large calanoids and euphausiids could have been consumed at different feedings, particularly if their vertical distributions overlapped with those of the fish at different times. Predation by pollock and herring on euphausiids in areas where these macrozooplankters were not collected suggests that the fish fed in a different area or at a different time.

Comparison of allopatric and sympatric aggregations

If competition occurs between sympatric species, one would expect that, given similar prey fields, the quantity or quality of prey consumed would improve when fish are allopatric. If such changes are prolonged, growth or survival differences are probable. We found intraspecific changes in diet composition and food quantity for both species from allopatric to sympatric aggregations, and weak interspecific differences in feeding between sympatric fish. Both species of fish avoided small calanoids whether a competitor was present or not, but the proportional use of energetically advantageous taxa (large calanoid, euphausiid, and hyperiid prey) and the selection for large calanoids were lower in the presence of competitors. Comparisons of sympatric fish showed that pollock ate proportionally more high energy-producing prey (euphausiid biomass, hyperiid frequency, large calanoid numbers) and selected *Metridia* spp. more strongly than herring. Overall, however, high diet overlap between allopatric and sympatric fish of either species in autumn indicated little change in diet composition owing to sympatry. Because both species of sympatric fish ate less total prey than allopatric fish, quantity, rather than quality of prey decreased in the presence of competitors or perhaps as a density-dependent response. Drastic diet shifts were demonstrated in one study involving fish removal; planktivorous species became benthivorous when their competitors were removed from a lake (Persson and Hansson, 1999). In our study, however, although the intraspecific comparisons (allopatric-sympatric) were within month, they differed by depth, time, or region; the interspecific comparisons of allopatric fish were a month apart in different years. Many authors have found differences in zooplankton prey fields on these scales[10] (e.g. Springer et al., 1989; Celewycz and Wertheimer, 1996), making it difficult in our study to separate the effects of sampling differences from those due to competition on diet and feeding.

Seasonal feeding

We observed the highest frequency of empty stomachs for both species in the November 1994 samples; empty stomachs, however, were more frequent for herring than for pollock. Similarly, in other seasonal studies, the proportion of empty stomachs in YOY herring peaked in winter (Foy and Norcross, 1999a; Foy and Paul, 1999), but empty stomachs were never observed among YOY pollock, even though stomachs were least full in December.[12] As zooplankton biomass declined between the winter months of October and February, the feeding response and whole body energy content of herring also declined (Foy and Paul, 1999). The herring relied on stored energy to overwinter (Paul et al., 1998a; Paul and Paul, 1998), but pollock are thought to allocate energy from year-round feeding for somatic growth (Paul et al., 1998b).

We also observed declines in zooplankton coincident with feeding declines in autumn; conversely, a greater food supply of small calanoids supported trends toward more intensive feeding in summer. Feeding on small calanoids in summer could have been density dependent because this taxon was found to be most prominent in the diets when it was more abundant in zooplankton samples. Juvenile Baltic herring exhibited a similar functional response to greater food densities (Arrhenius and Hansson, 1999). However, decreased feeding on small calanoids is more likely related to fish size and energy requirements. Both species (especially pollock in November 1994) were larger in autumn when they ceased consuming small calanoids, and both species avoided them more often. Larger prey are more efficient sources of energy for larger fish, if available. The larger autumn fish were probably better able to prey on late stages of macrozooplankton than the summer fish (Merati and Brodeur, 1996; Haegele, 1997; Kamba, 1977), in synchronism with a seasonal difference in their abundance and availability[13] (Bollens et al., 1992a; Stone and Jessop, 1994; Incze et al., 1997). A shift to larger prey is consistent with a decrease in prey numbers without a change in prey weight because fewer large prey are needed for equivalent weight. The biomass percentages of large prey consumed (large calanoids and euphausiids) and the numeric percentages of small prey consumed (larvaceans) both increased in autumn compared with summer (see also Foy and Norcross, 1999a). Ontogenetic changes and size-selective feeding have been reported by others, as well, for juvenile herring (e.g. Raid, 1985; Munk, 1992; Arrhenius, 1996) and juvenile pollock (Grover, 1991; Brodeur, 1998).

In conjunction with the seasonal diet transition, total prey number and most other measures of quantity were lower in autumn than in summer, particularly for herring. Autumn declines in juvenile pollock feeding rates were also observed in a study in Southeast Alaska in conjunction with a switch from small prey to larger prey.[12] Similarly, feeding declined seasonally for Atlantic herring (de Silva, 1973), Baltic herring (Arrhenius and Hansson, 1999), and Pacific herring (Foy and Norcross, 1999a). In our study, pollock consumed well above maintenance ration (0.30 %BW at 7.5°C; Smith et al., 1986) in all time periods, but, the low prey %BW of herring in autumn 1994 could indicate starvation, given a ration of 1.3–3.6 %BW at 6.2–8.7°C (de Silva and Balbontin, 1974; Arrhenius and Hansson, 1994). Continued feeding and better fish condition late in the year are advantageous for survival through the extreme conditions of winter, and relate to the species different strategies for overwintering.

Declines in feeding with season were also indicated by increased diet overlap between sympatric species in November compared with overlap in October, which suggests a density-dependent convergence of feeding with declining prey resources in late autumn. For sympatric fish in autumn, lower diet overlap occurred when pollock were more selective for large calanoids and ate proportionally more euphausiids, and the herring selected larvaceans (October 1995); greater dietary overlap in autumn occurred when the species ate similar percentages of these taxa and small calanoids (November 1994).

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[13] Mooney, J. R. 1999. Distribution, energetics, and parasites of euphausiids in Prince William Sound, Alaska. Unpubl. MS thesis, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11122 Glacier Hwy., Juneau, AK 99801, 172 p.

Despite larger size in autumn, energy requirements of fish are lower during this period of less abundant food due to environmental changes. In PWS, surface temperatures declined from approximately 12°C in summer, to 10°C in October 1995, and 7–8°C in November 1994.[2] Temperatures below the thermocline were stable at 5–6°C, but thermocline depth varied seasonally, between approximately 40–60 m in summer and 50–90 m in either autumn.[3,4] Additional seasonal changes in the environment have been reported by Stokesbury et al. (1999). From summer to winter in PWS, surface waters inside bays changed from being colder to being warmer than surface waters outside bays. Such environmental temperature changes in both the vertical and horizontal planes may influence the distribution of pollock and herring, their energy budgets (Smith and Paul, 1986), and the seasonal distribution and abundance of their prey.

Fish can conserve energy during times of reduced prey by altering behaviors to maximize prey searching or to decrease metabolic costs. For example, fish can alter school cohesiveness (Brodeur and Wilson, 1996b; Stokesbury et al., 1999), restrict their movement, or shift in residence to deeper water with colder temperatures (Sogard and Olla, 1996; Ciannelli et al., 1998). However, they may do so at the risk of increased predation or decreased light for feeding. Herring are primarily visual feeders, requiring minimum light levels to feed (Blaxter and Hunter, 1982) but may also filter feed in low light (Batty et al., 1986; Stone and Jessop, 1994). They can respond to prey distributions correlated with thermocline depth (Fossum and Johannessen, 1979) and may shift feeding during the day to depths where light is not restrictive even if prey are concentrated elsewhere (Munk et al., 1989). Similarly, the vertical distribution of juvenile pollock is affected by the relative availability of food and by numerous other factors that affect feeding conditions: predator presence, light, turbidity, pressure, temperature, size, and metabolic requirements (Bailey, 1989; Olla et al., 1996; Sogard and Olla, 1996; Ciannelli et al., 1998). Juvenile pollock avoided light more and avoided cold water less with growth, especially under conditions of low zooplankton (Olla et al., 1996). Both juvenile pollock (Smith et al., 1986) and YOY herring (de Silva and Balbontin, 1974; Arrhenius and Hansson, 1994) decreased prey consumption at colder temperatures; pollock also had lower maintenance rations and grew more rapidly under conditions of low food with colder temperatures (Smith et al., 1986). Competition with pollock for food could compromise the winter survival of YOY herring by limiting the accumulation of their energy stores.

Research showing major differences in the nutritional quality of forage species consumed by piscivores has sparked interest in their trophic interactions. Pollock lipid content was low compared with that of herring, but unlike herring, was not correlated with size (Anthony and Roby, 1997; Payne et al., 1999; Anthony et al., 2000). However, only a few studies have examined the energetic consequences of feeding on different prey for juvenile pollock (e.g. Smith et al., 1986; Davis and Olla, 1992; Ciannelli et al., 1998) and herring (e.g. de Silva and Balbontin, 1974; Arrhenius and Hansson, 1999). Because prey differ in nutritional composition, caloric density (Ikeda, 1972; Lee, 1974; Deibel et al., 1992; Davis et al., 1998), size, mobility, and behavior, their relative abundance is not the only factor of importance. For example, larvaceans are a highly visible taxon (Bailey et al., 1975) with caloric value per unit weight similar to that of crustacean zooplankton even though they are gelatinous (Davis et al., 1998), but many more larvaceans must be consumed to accumulate the equivalent calories obtained from the crustaceans. Davis and Olla (1992) showed in a controlled experiment that larval pollock growth, behavior, and lipid concentration were affected by the nutritional quality of prey. In a field study, herring diets had the highest energy density of all in May, when large calanoids were the most important taxon (Foy and Norcross, 1999a); however, they were not examined from late fall—a period for which our study showed that euphausiids were the prominent prey and others have shown they contain higher energy density compared with earlier times of the year.[13] If sympatry induces feeding on prey of lesser nutritional quality for extended periods because of interference competition, fish growth and survival could be affected.

For sympatry to occur, the distribution of juvenile walleye pollock and Pacific herring must overlap in three dimensions: time (seasonal and diel), and both horizontal and vertical space. These species have different life histories (Smith, 1981; Lassuy, 1989) and patterns of movement change ontogenetically, suggesting that spatial overlap is likely to vary. YOY herring generally school near the bottom along shore during the day, then move up to the surface at dusk and disperse (Blaxter and Hunter, 1982; Lassuy, 1989; Haegele, 1997). Early YOY pollock stay principally in surface water above the thermocline, perform a diel vertical migration (DVM), and disperse or move inshore at night; depth distribution increases from summer to autumn[12] and with ontogeny (e.g. Nakatani, 1988; Karp and Walters, 1994; Olla et al., 1996). Stokesbury et al. (1999) found that herring and pollock were generally depth stratified but that both were aggregated in bays in July and October. Rather than having a strong species association, they may simply have an affinity for the same habitats at some points in their life histories (Brodeur and Wilson, 1996a). Data collected monthly in PWS in 1994 (Willette et al., unpubl. data) showed that, after May, >50% of juvenile herring sets also caught juvenile pollock, and after July, >50% of juvenile pollock sets also caught juvenile herring. Willette et al. (1997) noted that diet overlap was more than twice as great for pollock and herring from sympatric sites than for these species from allopatric sites in late summer. These patterns suggest that sympatry and feeding competition increase from spring through summer.

Our study is the first to examine the feeding interactions of juvenile walleye pollock and Pacific herring. Its limited scope, because it was designed for other primary purposes, necessitates a primarily descriptive approach. Differences that we observed in fish feeding could relate to variable sampling intensity, limited fish sample sizes, gear limitations for certain prey taxa, spatial variation in prey, or differences in fish depths. Nonetheless, the similarity of dietary requirements between YOY pollock and herring could induce competition for limited food when these fish co-occur during periods or in places of low food availability, particularly late autumn and winter. Stokesbury et al. (1999) showed spatial variation in growth of YOY herring among widespread bays in PWS and surmised that density-dependent interspecific competition for food was one variable that affected growth rates; likewise, Paul and Paul (1999) showed interannual and spatial variations in size and energy content of YOY pollock from PWS and speculated that prey production and delivery contributed to the differences. The densities of planktivorous predators may exceed the carrying capacity of bays where walleye pollock and Pacific herring are sympatric, adding interspecific competition to the factors limiting growth. More studies comparing spatial and temporal patterns of distribution, abundance, and feeding are needed to clarify the extent and frequency of interactions between YOY pollock, herring, and other forage species and the impact of changes in prey and climate on these interactions.

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Age and growth of the bigeye tuna, *Thunnus obesus,* in the western Pacific Ocean

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Abstract—Bigeye tuna (*Thunnus obesus*) age and growth studies were last conducted in the western Pacific in 1967 and no study has ever attempted to age bigeye tuna from this area by using dorsal spines. The objective of our study was to estimate bigeye tuna age and growth rate in the western Pacific based on counts of growth rings on sections of the first dorsal spine. Length and weight data, and the first dorsal spine from bigeye tuna in the Tungkang (southwest of Taiwan) fish market were collected monthly from February 1997 to January 1998. In total, 1149 specimens were collected. The fork lengths of individuals ranged from 45.6 to 189.2 cm. Cross sections from dorsal spines were taken and examined under a dissecting microscope equipped with an image analysis system. The monthly percentage of specimens having a terminal translucent zone indicated that growth rings formed once a year; therefore, the age of each fish was determined from the number of visible growth rings. Von Bertalanffy growth parameters were estimated for males, females, and both sexes combined. There was no significant difference between males and females. The parameter estimates for the combined sexes were asymptotic length (L_∞) = 208.7 cm, growth coefficient (K) = 0.201/yr, and age at zero length (t_0) = –0.9906 yr.

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Fish. Bull. 99:502–509 (2001).

Bigeye tuna (*Thunnus obesus* Lowe, 1839) are a commercially important species of tuna inhabiting the warm waters of the Atlantic, Indian, and Pacific oceans. They are found across the entire Pacific between northern Japan and North Island of New Zealand in the west and from 40°N to 30°S in the east (Calkins, 1980; Matsumoto, 1998). Adult bigeye tuna are caught mainly by longlines, but substantial numbers of juveniles are taken by purse seines.

Taiwanese distant water tuna longline fleets have operated throughout these three oceans since the late 1960s targeting albacore. In the early 1980s, the Taiwanese began equipping their longliners with very cold (below –55°C) freezers and deep longlines in the Indian and Atlantic oceans, which allowed them to target bigeye tuna for the lucrative sashimi market in Japan. In the western Pacific, the Taiwanese offshore longline fleets, based in domestic (Tungkang mainly) and foreign fishing ports, have landed more bigeye tuna than in the past.

Growth studies of Pacific bigeye tuna conducted in the 1950s and 1960s were based either on increments between modal points in size-composition data (Iversen, 1955; Shomura and Keala, 1963; Yukinawa and Yabuta, 1963; Kume and Joseph, 1966; Suda and Kume, 1967) or on the number of annual markings (annuli) on scales (Nose et al., 1957; Yukinawa and Yabuta, 1963). Recently, Hampton and Leroy[1] and Matsumoto (1998) presented preliminary results from growth studies based on otolith increment counts. No previous study had aged Pacific and Indian bigeye tuna from dorsal spines, although a few age determination studies existed for Atlantic bigeye tuna (Gaikov et al., 1980; Draganik and Pelczarski, 1984; Delgado de Molina and Santana, 1986; Alves et al., 1998). Accurate age structure of stocks is essential for stock assessment and fishery management. Our study provides estimates of the age and growth rate of bigeye tuna in the western Pacific from growth rings on sections of the first dorsal spine.

Materials and methods

Fork length (in cm), weight (in kg), and sex were determined for bigeye tuna caught by Taiwanese offshore longliners in the fishing area from 23°N to 0°N and 110°E to 140°E (Fig. 1[2]) and sold at the Tungkang fish market between February 1997 and January 1998. In addition, a total of 1149 first dorsal spines were collected. Three cross sections were taken along the length of each spine above the condyle base (Fig. 2A) with a low-speed “ISOMET” saw

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[1] Hampton, J., and B. Leroy. 1998. Note on preliminary estimates of bigeye growth from presumed daily increments on otoliths and tagging data. Working paper 18, eleventh meeting of the standing committee on tuna and billfish, Honolulu, Hawaii, USA, 30 May–6 June 1998, 3 p. Oceanic Fisheries Programme, Secretariate of the Pacific Community, B.P.D5, 98848 Noumea, New Caledonia.

[2] Yang, R. T., R. F. Chung, and C. L. Chang. 1982. Taiwanese offshore tuna longline fishery. Part I: fishing ground, fishing season, and fishing condition. Spec. Rep. 36, 6 p. [In Chinese with English abstract.] Insitute of Oceanography, National Taiwan University, no. 1, sec 4, Roosevelt Rd, Taipei, 106 Taiwan.

(model no. 11-1280) and diamond wafering blades. Sections ranging from 0.8 to 1.0 mm thick (Fig. 2B) were examined with a dissecting microscope (model: Olympus SZH-ILLD) with transmitted light. Images of the dorsal spine sections were captured by using an image analysis software package, a CCD (charged coupled device) camera, and a high-resolution computer monitor. Translucent rings on the section images were counted by two readers independently. When ring counts disagreed, images were read again by both readers simultaneously, and any questionable spines were discarded.

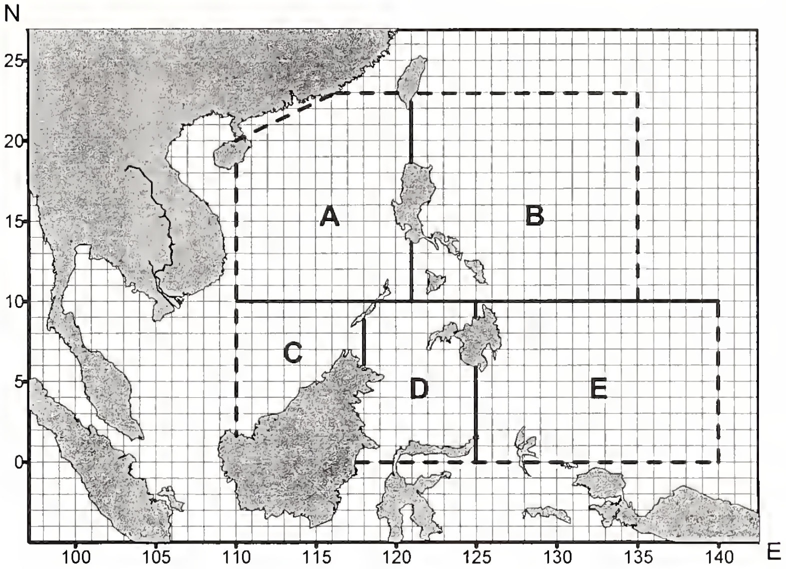

Figure 1

Fishing areas of the Taiwanese offshore tuna longline fishery in the western Pacific Ocean (Yang et al.[2]).

Spine sections as the structure to estimate age have the advantage of requiring easy sampling and easy reading (the growth rings stand out clearly), and samples are easily stored for future reexamination (Compeán-Jimenez and Bard, 1983). However, early growth rings may be lost in larger specimens because of increased size of the vascularized core in the spine. Accordingly, we estimated the number of lost (obscured) rings from observations of their position and number in spines from young specimens as has been done for little tunny (*Euthynnus alletteratus*) (Cayré and Diouf, 1983), eastern Atlantic bluefin tuna (*Thunnus thynnus*) (Compeán-Jimenez and Bard, 1983), and Pacific blue marlin (*Makaira nigricans*) (Hill et al., 1989).

Age was determined from the translucent rings, assuming that two rings are formed each year—a translucent (light colored) ring formed during the slower growth period and an opaque (dark colored) ring formed during the fast growth period. This assumption was validated by observing a translucent or opaque edge on the dorsal-spine sections and a monthly variation in the number of translucent edges (Antoine et al., 1983).

Distance between the center of the dorsal spine and the outer edge of each annual ring was measured in microns with the software package after calibration against an optical micrometer. The center of the spine was estimated by following Cayré and Diouf (1983) (Fig. 2B). Distances (d_i) were then converted into radii (R_i) by following González-Garcés and Fariña-Perez (1983).

The relationship between fork length (FL) and dorsal spine radius (R) was modeled by a linear equation (Zar, 1999). Fork length was then back-calculated for each ring with the formula (Lee, 1920)

$$FL_i = a + \frac{(FL-a)R_i}{R},$$

where FL_i = predicted fork length of the fish corresponding to age or ring i in cm;
a = ordinate in the origin of the equation $FL = a + bR$;

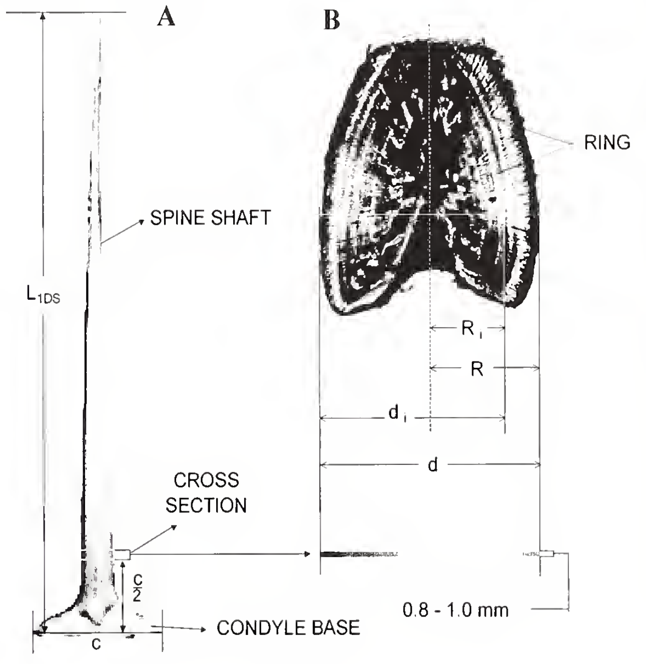

Figure 2

First dorsal spine and the site of cross section (**A**) and the cross section showing annual rings and measurements taken (**B**) for age determination of the western Pacific bigeye tuna (c =width of condyle base; L_{1DS}=length of the first dorsal spine; R=radius of spine; R_I=radius of ring i; d=diameter of spine; d_I=diameter of ring i).

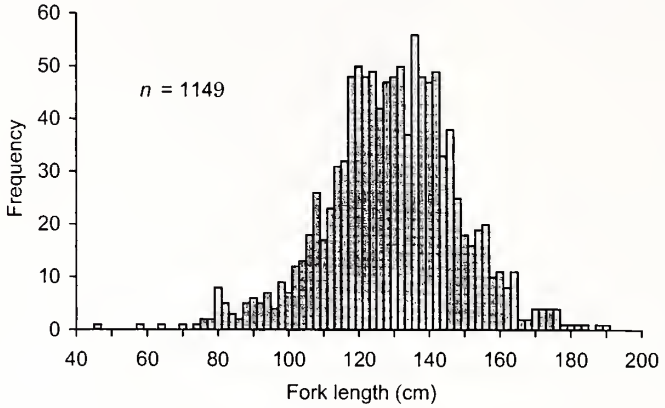

Figure 3

Length-frequency distribution for the western Pacific bigeye tuna sampled at Tungkang fish market from February 1997 to January 1998.

FL = observed fork length of the fish in cm;
R_i = radius of the ring calculated as the average value observed in ring i (Fig. 2B); and
R = dorsal spine radius.

Back-calculated fork lengths were used in Ford-Walford (Gulland, 1983) and nonlinear (Ratkowsky, 1983) methods to fit the von Bertalanffy growth function (VBGF) and to obtain vital parameters by sex. Analysis of the residual sum of squares (ARSS) was employed to compare the VBGF between sexes (Ratkowsky, 1983; Chen et al., 1992).

Weight was related to fork length by using the power function, and analysis of covariance (ANCOVA) (Steel, 1980; Zar, 1999) was conducted to examine differences between sexes.

Results

Spines from 1149 specimens ranging in size from 45.6 to 189.2 cm FL were examined (Table 1, Fig. 3). There was 90% agreement between the readers' counts of growth rings and second readings improved this agreement to 95.6%, which resulted in discarding 51 specimens from analysis.

The relationship between FL (cm) and weight (kg) is shown in Figure 4. The ANCOVA indicated no significant difference between males and females ($P>0.05$); thus the FL-W relationship with sexes combined was expressed as

$$W = 3 \times 10^{-5} FL^{2.9278} \qquad (r^2=0.97, n=856).$$

The relationship of first dorsal spine lengths (L_{1DS}) and FL was (Fig. 5)

$$FL = 6.9367 L_{1DS} + 6.6667 \qquad (r=0.94, n=567).$$

The trend of the monthly percentages of terminal translucent edges (Fig. 6) suggested that the period from February to September was the long period of inhibited growth (translucent edge). From October to November, growth appeared to resume (opaque edge) and later, from December to January, a new translucent edge appeared; indicating the formation of one growth ring per year.

Given the significant linear relationship between the dorsal spine radius and fork length ($FL=26.455R + 19.916$, $r=0.94$, $n=1098$), we used spine measurements to back-

Table 1

Sample sizes, ranges of fork lengths (FL, cm), and sampling months and areas of bigeye tuna from the western Pacific Ocean. A, B, C, D, and E denote areas in Figure 1.

Month	Sampling area	Sample size	Minimum FL	Maximum FL
Feb 1997	A	80	70	174.5
Mar 1997	A	104	64	169.5
Apr 1997	B	70	101.3	171
May 1997	B	54	83.8	157.4
Jun 1997	B	71	75.5	162.8
Jul 1997	B, D	131	72.2	165.6
Aug 1997	E	94	78.5	187.7
Sep 1997	E	98	45.6	189.2
Oct 1997	A, C	115	86.5	176.6
Nov 1997	A, C	116	89.6	161.1
Dec 1997	C	123	104.6	162.1
Jan 1998	A	93	88.7	159.1
Total		1149	45.6	189.2

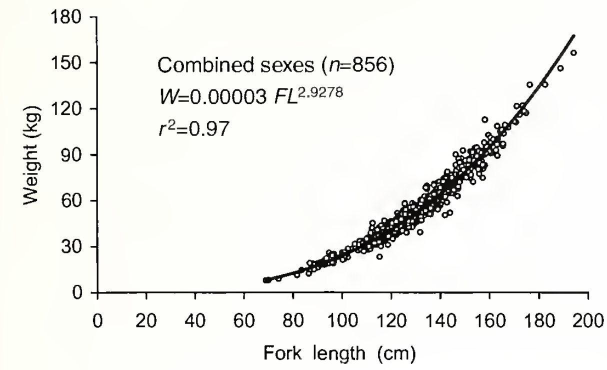

Figure 4

Relationships between weight and fork length of the western Pacific bigeye tuna sampled at Tungkang fish market.

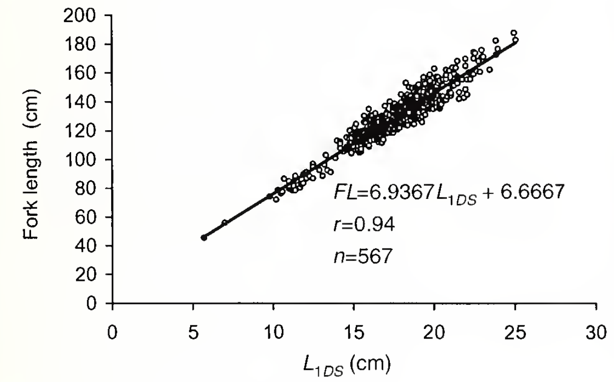

Figure 5

Relationship between fork length and length of the first dorsal spine (L_{1DS}) of the western Pacific bigeye tuna sampled at Tungkang fish market.

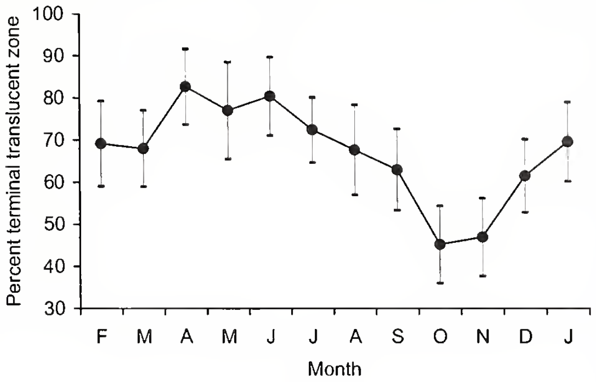

Figure 6

Monthly variation in percentage of the western Pacific bigeye tuna with a terminal translucent zone in dorsal spine sections, February 1997 to January 1998.

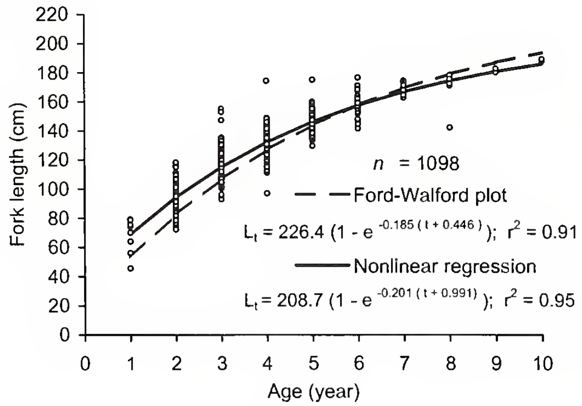

Figure 7

Comparison of the growth curve obtained by the Ford-Walford plot with the growth curve obtained by nonlinear regression method for the western Pacific bigeye tuna.

calculate the fork lengths of previous ages. The mean back-calculated fork lengths for the first 10 years of life for the western Pacific bigeye tuna are given in Table 2.

Parameters of the VBGF estimated by the Ford-Walford method for males, females, and sexes combined are shown in Table 3. Growth was not significantly different between sexes (ARSS, F=1.98; df=3, 452; P>0.05); the pooled growth curve is shown in Figure 7. VBGF parameters computed by nonlinear regression are also shown in Table 3 and Figure 7. Length-at-age of bigeye tuna estimated by nonlinear regression is larger (up to age 6 years) than that estimated by the Ford-Walford method.

Discussion

Available genetic information supports the hypothesis of a single bigeye stock in the Pacific Ocean (Hampton et al., 1998; Grewe and Hampton[3]). Although the fishing area of the Taiwan fleet and thus the sampling area of bigeye tuna used in our study was limited to a small area of the western Pacific, our results may be representative of bigeye tuna throughout the Pacific Ocean.

Monthly variation in percent terminal translucent edges in our study suggested the formation of growth rings once a year. Ehrhardt et al. (1996) attributed the narrow, trans-

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[3] Grewe, P. M., and J. Hampton. 1998. An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on mitochondrial DNA and DNA microsatellite analysis. University of Hawaii, Joint Institute for Marine and Atmosphere Research Contribution 98-320, 29 p. Pelagic Fisheries Research Program, University of Hawaii at Manoa, 1000 Pope Road, Honolulu, HI 96822.

Table 2

Observed and back-calculated mean fork length (FL, cm) at age for the western Pacific bigeye tuna, *Thunnus obesus*. ("—" means there were no data owing to vascularization at core area). Numbers in normal print represent the mean back-calculated fork lengths; numbers in parentheses represent the number of specimens for which the specified ring was readable.

Age		Observed mean FL	Annulus number									
(yr)	*n*	(cm)	I	II	III	IV	V	VI	VII	VIII	IX	X
1	8	67.9	57.0 (8)									
2	79	93.7	50.0 (29)	80.8 (79)								
3	329	115.3	52.4 (69)	85.8 (265)	102.9 (329)							
4	413	131.7	51.9	82.7 (19)	107.1 (162)	121.2 (384)	(413)					
5	188	145.9	—	80.9 (14)	112.1 (77)	122.8 (184)	135.8 (188)					
6	59	158.4	—	—	115.5	126.6 (3)	138.2 (36)	149.5 (58)	(59)			
7	11	169.3	—		119.5 (1)	130.1 (5)	142.6 (9)	152.1 (11)	161.8 (11)			
8	6	174.7	—	—	—	123.8 (1)	139.6 (5)	150.8 (6)	161.0 (6)	172.1 (6)		
9	3	178.5	—	—	—	121.0 (1)	129.2 (2)	142.0 (2)	154.2 (3)	169.2 (3)	174.2 (3)	
10	2	188.5	—	—	—	—	138.2 (1)	148.4 (2)	162.5 (2)	174.1 (2)	180.6 (2)	186.2 (2)
Total	1098		(125)	(520)	(794)	(640)	(263)	(80)	(22)	(11)	(5)	(2)
Weighted back-calculated mean FL (cm)			52.1	83.9	105.9	122.0	136.6	149.8	160.6	171.7	177.4	186.2
Growth increment (cm)			—	31.9	21.9	16.2	14.6	13.1	10.9	11.1	5.7	8.7

Table 3

Growth parameters obtained by the Ford-Walford plot method and the nonlinear regression method for the bigeye tuna from the western Pacific Ocean.

	Ford-Walford plot				Nonlinear regression
Parameter	Male	Female	Pooled	Total[1]	Total[1]
n	278	180	458	1098	1098
K	0.1789	0.191	0.1842	0.185	0.2011
L_∞	220.6	211.4	216.1	226.4	208.7
t_0	−0.5566	−0.4592	−0.5266	−0.4465	−0.9906

[1] Male, female, and sex-unknown combined.

lucent rings to slower growth periods, whereas the wide, opaque rings were attributed to periods of fast growth. The spawning season of bigeye tuna in the western Pacific is between February and September and peaks from March to June (Sun, et al.[4]), the period that we found to coincide

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[4] Sun, C. L., S. L. Chu, and S. Z. Yeh. 1999. Note on reproduction biology of bigeye tuna in the western Pacific. SCTB12/WP/BET-4, 6 p. Twelfth meeting of the standing committee on tuna and billfish; Tahiti, French Polynesia, June 14–23, 1999. Oceanic Fisheries Programme, Secretariate of the Pacific Community, B.P.D5, 98848 Noumea, New Caledonia.

Table 4

Comparisons of estimates of parameters of von Bertalanffy growth function for the Pacific bigeye tuna by various authors. Partly reproduced from Table 10 of Shomura (1966).

			Parameters				
Area	Investigator(s)	Method of analysis	L_∞ (cm)	K	t_0	Size range of fish (cm)	Comments
Pacific-wide, north of 10°S	Nose et al. (1957)	Scales	195.2	0.106	–1.128	Mean observed length for 58–109	Parameters based on mean observed length by age provided by authors
Western North Pacific (north of 2°N and west of 180°)	Yukinawa and Yabuta (1963)	Size frequency	257.5	0.156	–0.107	Modal sizes estimated to be 65–150	Parameters computed from data provided by authors
Pacific (north of 10°S)	Yukinawa and Yabuta (1963)	Scales	215	0.10412	0.0010995	Estimate 51–160	Authors' values; time (t) in half-year units
Pacific (north of 10°S)	Yukinawa and Yabuta (1963)	Scales (same basic data as above)	213.1	0.212	0.017		Parameters computed by graphic method from data provided by authors
Central Pacific (Hawaiian Islands)	Shomura and Keala (1963)	Size frequency (males)	196.7	0.267	–0.929		Authors' values
Central Pacific (Hawaiian Islands)	Shomura and Keala (1963)	Size frequency (females)	183.0	0.316	0.718		Authors' values
Eastern Pacific (east of 130°W and between 10°N and 25°S)	Kume and Joseph (1966)	Size frequency	186.95	0.095	2.11	39–209	Authors' values; time (t) in quarter-year units
Pacific area	Suda and Kume (1967)	Size frequency	214.8	0.2066	0.0249		
Western Pacific	Hampton and Leroy (1998)	Otolith and tagging	165.3	0.3732	0.3420	25–175	Preliminary
Eastern Pacific	Matsumoto (1998)	Otolith	—	—	—	33.4–57.9	Author estimated only the lengths of 40 and 55 cm at ages of 0.5 and one year, respectively; preliminary.
Western Pacific	This study	Spine	208.7	0.2011	–0.9906	45.6–189.2	

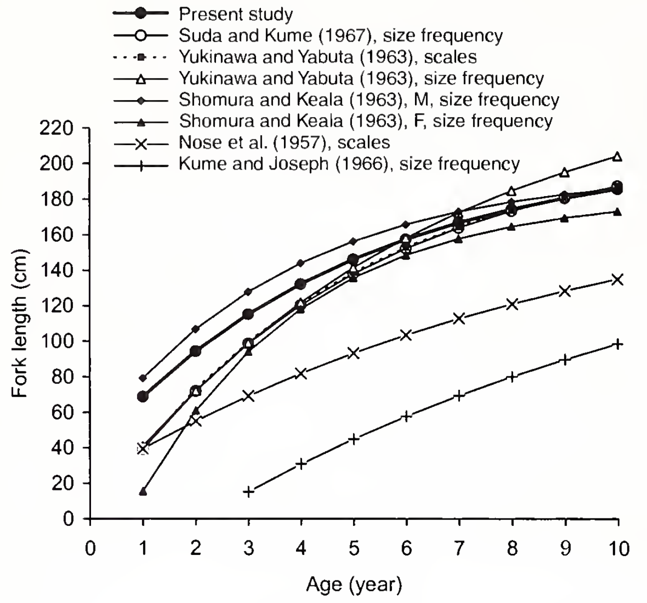

Figure 8

Growth curves of Pacific bigeye tuna estimated by different authors.

with the slow-growth period indicated by the narrow and translucent rings. Similar findings have been reported for skipjack tuna (Antoine et al., 1983), bigeye tuna (Gaikov et al., 1980), and swordfish (Ehrhardt, 1992; Tserpes and Tsimenides, 1995). Our efforts only partially validate fish age; complete validation requires either mark-recapture data or the study of known-age fish in the population (Beamish and McFarlane, 1983; Prince et al., 1995; Tserpes and Tsimenides, 1995).

We estimated the parameters of the VBGF by using the Ford-Walford and nonlinear methods and found that the nonlinear method had a better fit (r^2=0.95) than the Ford-Walford method (r^2=0.91). Comparisons of our VBGF parameters with previous studies (Fig. 8, Table 4) showed similar results to those of Yukinawa and Yabuta (1963) who used scales and to those of Suda and Kume (1967) who also used Pacific samples of bigeye tuna. The values of t_0 differed because different aging techniques were used. Following the suggestion of Gallucci and Quinn (1979), Vaughan and Kanciruk (1982), and Hanumara and Hoenig (1987) that Ford-Walford and other linear methods be replaced by nonlinear fitting techniques; we propose using parameters of VBGF estimated by the nonlinear method (Table 3) for description of age and growth for the western Pacific bigeye tuna.

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Nocturnal occurrence of the swimming crab *Ovalipes punctatus* in the swash zone of a sandy beach in northeastern Japan

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Swimming crabs belonging to the genus *Ovalipes* (Crustacea: Brachyura: Portunidae) are distributed worldwide along sandy coastlines of subtropical and temperate waters (Stephenson and Rees, 1968). They are extremely well adapted to life on sand, with their strong swimming and burrowing abilities (Brown and McLachlan, 1990). Crabs of this genus are voracious carnivores and significant predators of commercially important mollusks on sandy beaches (Du Preez, 1984; Brown and McLachlan, 1990). Recently, interest in their importance as a possible fisheries resource has increased (Sasaki and Kawasaki, 1980; Wear and Haddon, 1987).

Ovalipes punctatus (De Haan) is common in the coastal waters of Japan and China. It is found on sandy bottoms below the intertidal zone, but peak abundance is at 5–60 m. This species has an ontogenetic migration into deeper water (Kamei, 1976; Sasaki and Kawasaki, 1980). Sasaki and Kawasaki (1980) investigated the life history of *O. punctatus* in Sendai Bay (100 km south of the present study site), on the Pacific coast of northeastern Japan. Female crabs spawn from mid-September until mid-November in offshore water 40–60 m deep. The incubation period of the eggs is about 20 days. After a planktonic phase, the larvae settle on sandy bottoms in March and April. Settled crabs of both sexes grow to carapace widths (CW) of 50–65 mm in the first year. In the second year, females and males grow to 70–90 and 80–85 mm CW, respectively. The life span of this crab is 2 to 2.5 years. The minimum size at maturity is estimated to be 45–50 mm CW (Sasaki and Kawasaki, 1980), but the spawning population consists mainly of 2-year-old crabs of 75–80 mm CW. However, knowledge of the diet and feeding habits of this species has been extremely limited until now.

Ovalipes species, including *O. punctatus*, generally live below the intertidal zone. However, they are often found in the intertidal zone, especially at night (McLachlan et al., 1979; Brown and McLachlan, 1990). McLachlan et al. (1979) suggested that this occurrence is related to feeding, but details are still unknown. During the course of an ecological study of the sandy beach in Otsuchi Bay, we frequently found *O. punctatus* in the swash zone at night. This note examines the significance of the swash zone in the life history of the swimming crab *O. punctatus* in relation to feeding and molting.

Materials and methods

The study was conducted at Koshirahama Beach, in Otsuchi Bay, on the Pacific coast of northern Honshu, mainland Japan, in September of 1994 and August of 1995 (Table 1). The beach is about 120 m long, bounded on both ends by rocky shores, and is categorized as a "sheltered beach" according to McLachlan's (1980) rating scheme. Mean depth at 20, 50, and 100 m from the shoreline is 1.6, 3.5, and 5.5 m, respectively. The beach has a 1:13 (rise: run) slope and median particle diameter of the sediment is 260 µm. Other details of the study site are given in Takahashi and Kawaguchi (1995).

Crabs were sampled with a beach seine 1.1 m high by 8.3 m wide with 1.0-mm mesh. The net was hauled parallel to the shoreline at a depth of ca. 0–1 m. Samples were taken along the entire shoreline of Koshirahama Beach. All crabs were preserved in 10% formalin, and the carapace width (CW), sex, maturity and molt stage of each individual were recorded. The foregut was removed and stored in 70% ethanol for dietary analysis. Sexual maturity of *O. punctatus* was assigned by size classes as defined by Sasaki and Kawasaki (1980): juvenile (<30 mm CW), immature (31–50 mm CW), or adult (>51 mm CW). Molt stages were based on the criteria of Drach and Tchernigovtzeff (1967) and Norman and Jones (1992): 1) soft, no calcification of the new exoskeleton; 2) early papershell, thin, flexible exoskeleton, easily depressed when touched; 3) late papershell, hard exoskeleton except for the branchiostegite region which is compressible; 4) intermolt, completely hard exoskeleton; and 5) premolt, teeth on chelae well worn and having a less rounded appearance compared with those in earlier stages, complete exocuticle developed beneath the exoskeleton.

The diet of *O. punctatus* was analyzed by using the points method and the percentage occurrence method (Williams, 1981; Wear and Haddon, 1987). The points method assesses diet composition in terms of both foregut fullness and estimated volume of food in the foregut. First, the relative degree of foregut fullness of each crab was estimated visually by using six ordered classes (empty=class 0; trace=class 1; 25%=class 2; 50%=class 3; 75%=class 4; full=class 5). A visual assessment of fullness was possible because, except for the gastric mill, the foregut of *O. punctatus* is like a thin-walled translucent bag.

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Table 1

Details of the sampling dates and times (JST) and results in the swash zone of Koshirahama Beach, Otsuchi Bay.

Date	Day or night	Sampling time (h)	No. of crabs collected
22 Aug 1995	Day	8:02	3
25 Aug 1995	Day	13:12	2
30 Aug 1995	Day	13:15	3
12 Sept 1994	Night	20:54	30
25 Aug 1995	Night	21:06	23
30 Aug 1995	Night	20:48	35

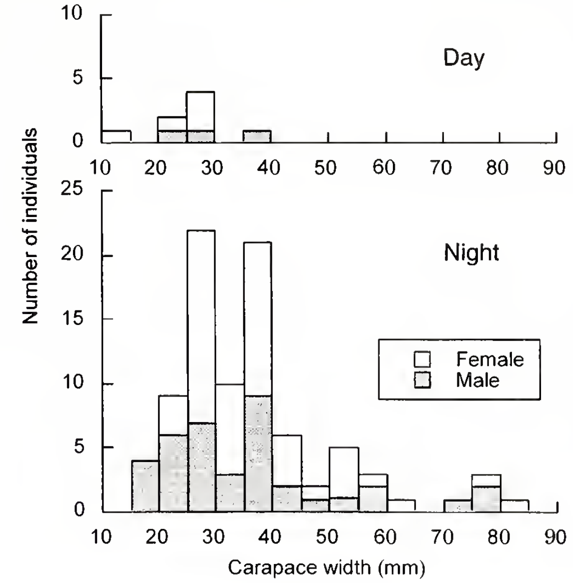

Figure 1

Histograms of carapace width for the swimming crab *Ovalipes punctatus* found in the swash zone of Koshirahama Beach, Otsuchi Bay, during the day and at night. All the specimens were collected in September 1994 or August 1995.

Food items were identified to the lowest possible taxon under a binocular dissecting microscope. The relative contribution of each prey category to the total volume of the foregut contents was assessed subjectively in the following way: a category representing 95–100% of the total contents was awarded 100 points; 65–95%, 75 points; 35–65%, 50 points; 5–35%, 25 points; 5% or less, 2.5 points; empty, 0 points. The points that each prey category received were weighted by multiplying by a factor that depended on the degree of foregut fullness, i.e. full = 1, 75% = 0.75, 50% = 0.5, 25% = 0.25 and trace = 0.02 (Wear and Haddon, 1987). The maximum and minimum weighted points possible for a single category in a single foregut were 100 (100 × 1.0) and 0.05 (2.5 × 0.02), respectively. The following percentages were calculated for each prey category (Williams, 1981):

Percentage points for ith prey = $\left(\sum_{j=1}^{n} a_{ij} \Big/ A\right)100$; and

Percentage occurrence for ith prey = $(b_i/N)100$,

where a_{ij} = the number of points for prey item i in the foregut of the jth crab;
A = the total points for all the crabs and all the prey items in all the foreguts examined;
N = the number of crabs examined with food in the foregut; and
b_i = the number of crabs with foreguts containing prey category i.

Results

Diel change in the swash zone occurrence pattern

A total of 96 crabs (41 males, 55 females), ranging from 12 to 80 mm CW, were collected from the swash zone of Koshirahama Beach (Fig. 1). No ovigerous females were collected.

Almost all specimens were collected at night (88 individuals, 92% of the total catch) and only 8 crabs were collected during the day (Fig. 1). Juvenile (35) and immature (39) crabs dominated the nocturnal catches, accounting for 40% and 44% of the total nocturnal catch, respectively (Table 2). Fourteen adult crabs were also caught at night, constituting 16% of the nocturnal samples (Table 2). Daytime catches consisted of 1 immature and 7 juvenile crabs (Table 2). On average, crabs caught at night (36 mm CW) were significantly larger than crabs caught during the day (25 mm CW) ($P<0.01$; t-test).

All crabs were in the intermolt or the papershell stages (early and late papershell); no soft-shelled and premolt crabs were collected. During the daytime, 7 out of 8 crabs were in the papershell stages and only one individual was classified as intermolt (Table 2). At night, 5 juvenile and 5 immature papershell crabs were collected, accounting for 14% and 13% of the total catch for each category, respectively (Table 2). Of the adults collected at night, 9 were in the papershell stages, accounting for 64% of the total adult crabs taken.

Feeding activity and diet composition

Because feeding habits of early and late papershell crabs are not different from those of intermolt stages in portunid crabs (Norman and Jones, 1992), analysis for foregut fullness and diet was conducted by pooling all molt stages. The percent frequency of each class of foregut fullness is shown separately for day and night in Figure 2. Although the daytime sample was small, 50% of the crabs had empty foreguts (Fig. 2), but at night over 70% of the

Table 2

Ovalipes punctatus caught in the swash zone of Koshirahama Beach, Otsuchi Bay, during the summers of 1994 and 1995. The numbers of each developmental stage and of papershell crabs caught during the day and at night are shown. No softshell and premolt crabs were collected.

Day or night	Developmental stage of crabs (CW)	Total number of crabs collected	Number of papershell crabs: Early papershell	Number of papershell crabs: Late papershell
Day	Juvenile (12–30 mm)	7	6	—
	Immature (31–50 mm)	1	1	—
	Adult (51–80 mm)	—	—	—
Night	Juvenile (12–30 mm)	35	4	1
	Immature (31–50 mm)	39	4	1
	Adult (51–80 mm)	14	3	6

Table 3

Percentage occurrence, points, and percentage points for the 12 categories of foregut content in 69 *Ovalipes punctatus* from Koshirahama Beach, Otsuchi Bay. The points express the dietary contribution in terms of foregut fullness and estimated volume of food items in the foregut (see text). Sand was excluded from the calculation of the percentage points.

Food item	Percentage occurrence	Points	Percentage points
Crustaceans			
Haustorioides japonicus	59	1437.5	26.1
Archaeomysis kokuboi	54	1287.5	23.4
Excirolana chiltoni	30	528.7	9.6
Crangon sp.	14	540.0	9.8
Crustacean fragments	42	1115.6	20.3
Other crustaceans	4	62.5	1.1
Mollusks			
Bivalves	6	41.3	0.7
Gastropods	1	25.0	0.5
Fishes			
Unidentified fish	3	5.0	0.1
Other items			
Unidentified organic matter	19	456.3	8.3
Algae	3	3.8	0.1
Sand	94	970.6	—

foreguts examined were more than half full (classes 3–5) and 7% had empty foreguts, which suggests that the crabs fed actively in the swash zone at night (Fig. 2).

When describing the diet of portunid crabs, Williams (1981) recommended including only individuals with foreguts more than 50% full (i.e. classes 3–5). Applying this criterion, we found 69 crabs (39 females, 30 males) for the diet analysis, 66 of which were collected at night. Because there was no significant difference in the diets by sex (chi-square test, $P>0.5$), the male and female data were combined. Forguts contained 11 prey categories plus sand; percent frequency of occurrence, total points, and the relative proportions of the diet components in terms of points are listed in Table 3. Although sand is not considered a part of the diet, it contributed more than 14% to the total points of the foregut contents.

Small crustaceans were frequent and predominant in the foreguts of *O. punctatus* (Table 3). The sand-burrowing amphipod *Haustorioides japonicus* was the most frequent prey, contributing 26.1% of the total points. Second most frequent prey was the sand-burrowing mysid *Archaeomysis kokuboi* (23.3% of the total points), followed by the sand-burrowing isopod *Excirolana chiltoni* (9.6%) and sand shrimps *Crangon* sp. (9.8%). Fragments that probably came from these crustaceans produced 20.3% of the total points (Table 3). The results show 90.2% of the total diet volume of *O. punctatus* consisted of crustacean prey. Mollusks and fish were not important in the diet (1.3% collectively).

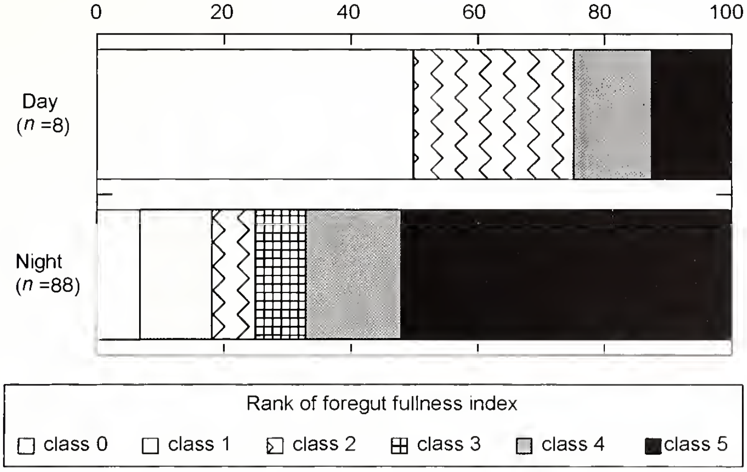

Figure 2

Difference in the foregut fullness index of the swimming crab *Ovalipes punctatus* in the swash zone of Koshirahama Beach, Otsuchi Bay between day and night. Class 0 = empty foregut, class 1 = trace items in foregut, class 2 = foregut 25% full, class 3 = 50% full , class 4 = 75% full, class 5 = full.

Discussion

Ovalipes punctatus occurred nocturnally in the swash zone of Koshirahama Beach, Otsuchi Bay. The nocturnal occurrence in the swash zone for congeners such as *O. trimaculatus* from South Africa (reported as *O. punctatus*, see Schoeman and Cockcroft, 1993) is often observed elsewhere (McLachlan et al., 1979; Brown and McLachlan, 1990). McLachlan et al. (1979) suggested that occurrence in the swash zone is related to feeding. Generally, *Ovalipes* species are active nocturnally (Caine, 1974; Du Preez, 1983). Our observations of foregut fullness and abundance support these findings. Furthermore, because peracarid crustaceans that live close to the sandy shoreline dominated the foregut contents (Kamihira, 1979; Takahashi and Kawaguchi, 1995; authors' pers. obs.), we concluded that part of the crab population migrates from deeper waters into the swash zone at night to exploit the crustacean fauna of sandy beaches.

Feeding in the swash zone is advantageous for the crabs because important prey, such as the amphipod *H. japonicus*, the mysid *A. kokuboi*, and the isopod *E. chiltoni*, are abundant in the swash zone of Koshirahama Beach. These species are considerably more abundant near the shoreline than other macrobenthic organisms that live in the offshore area (authors' pers. obs.). The peracarids, especially mysids and isopods, burrow into the sand during daytime, then emerge into water column in the swash zone at night (Takahashi and Kawaguchi, 1997; authors' pers. obs.) which makes them more vulnerable to predation by *O. punctatus* and fishes (Takahashi et al., 1999). The predominance of peracarid crustaceans in the foreguts of the crabs suggests that *O. punctatus* capture the peracarids effectively in the swash zone of Koshirahama Beach at night.

Species of *Ovalipes* are known as opportunistic, broad-spectrum predators. They are extremely versatile, feeding on both less active prey, such as mollusks, and on mobile animals, such as amphipods, mysids, isopods, and fish (Caine, 1974; Haefner, 1985; Wear and Haddon, 1987; Ropes, 1989; Stehlik, 1993).

The swash zone also serves as a refuge from predation. Species of *Ovalipes* (including congeneric species) are occasionally found in the guts of fish and decapod crustaceans (McDermott, 1983; Du Preez and McLachlan, 1984; Mitchell, 1984; Wear and Haddon, 1987; Stehlik, 1993). Cannibalism is a major cause of mortality in many portunid crabs during their early life stages; the importance of refuges has been emphasized as a factor regulating crab recruitment in natural populations (Hines and Ruiz, 1995). In estuarine ecosystems, habitats with structural complexity, i.e. seagrass beds, mussel beds, filamentous algae, are effective as refuges for juvenile crabs, whereas risk of predation is higher in open sand, the main habitat of *Ovalipes* (Wilson et al., 1987; Ryer et al., 1997, Moksnes et al., 1998). In sand habitat, small *Ovalipes* burrow deep by using reverse gill current to hide from predators during the day (Barshaw and Able, 1990).

Nocturnal emergence of *Ovalipes* makes them more vulnerable to cannibalism. In the swash zone of Koshirahama Beach at night, almost all the *O. punctatus* were juvenile and immature crabs. This finding suggests that small individuals avoid the deeper zone, where the risk of cannibalism is high. For blue crabs, *Callinectes sapidus*, shallow water areas function as refuges from predation when habitats have little structural complexity (Dittel et al., 1995; Hines and Ruiz, 1995). The swash zone is probably difficult for larger predators to penetrate and they risk being stranded. In addition, birds that exploit the swash zone are less active at night (Brown and McLachlan, 1990).

Softshell and premolt crabs were not collected in the swash zone; it appears that this zone may not be a molting ground owing to its dynamic characteristics. However, most diurnal and adult crabs in the samples were papershell crabs, which are also vulnerable to predation; the swash zone, therefore, may be important as a refuge for postmolt crabs.

In our study, juvenile and immature crabs dominated the population of *O. punctatus* in the swash zone during late summer. From the size of the crabs, these were first-year crabs born in the previous winter or spring, and therefore their occurrence in the swash zone may be limited to the summer and fall, when these juvenile and immature crabs appear (Sasaki and Kawasaki, 1980). A similar seasonal occurrence of juvenile *O. ocellatus* from July to October on exposed sandy beaches of the mid-Atlantic Coast of the United States has been reported in an inshore area (McDermott, 1983). The occurrence and behavior of *O. punctatus* in the swash zone suggest that this habitat is important as a feeding ground and a refuge from predation. Intertidal sand-burrowing peracarids, the major diet items of *O. punctatus*, also consume particulate organic matter and organisms in the swash zone (Kamihira, 1992; Takahashi and Kawaguchi, 1998). Thus by feeding in the swash zone, young *O. punctatus* transport some of the secondary production from the swash zone to the offshore area through their ontogenetic migration into deeper water.

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***Fishery Bulletin* 98(4):759–766**

Lindley, Steven T., Michael S. Mohr, and Michael H. Prager

Monitoring protocol for Sacramento River winter chinook salmon, *Oncorhynchus tshawytscha*: application of statistical analysis to recovery of an endangered species

Erratum 1: page 759, right column, line 10. The equation within the text should read

$$R_t = N_t / N_{t-3}.$$

Erratum 2: page 760. Equation 1 should read

$$t = \frac{\bar{r} - \rho_{goal}}{s / \sqrt{n}}.$$

Erratum 3: page 761. Equation 2 should read

$$\delta = \frac{\rho - \rho_{goal}}{\sigma / \sqrt{n}}.$$

***Fishery Bulletin* 99(1):49–62**

Gharrett, Anthony J., Andrew K. Gray, and Jonathan Heifetz

Identification of rockfish (*Sebastes* spp.) by restriction site analysis of the mitochondrial ND-3/ND-4 and 12S/16S rRNA gene regions

Erratum: page 56, line 5

Read "*S. melanops*" instead of "*S. maliger*" so that the sentence begins

"Similarly, the haplotypes of *S. melanops* and *S. flavidus* (subgenus *Sebastosomus*) were tightly clustered…".

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Seasonal occurrence and site-utilization patterns of adult tautog, *Tautoga onitis* (Labridae), at manmade and natural structures in lower Chesapeake Bay*

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Abstract—Ultrasonic transmitters were surgically implanted into adult tautog (n=27, 400–514 mm TL) to document seasonal occurrence and site utilization at four sites situated within known tautog habitat near Cape Charles, Virginia, in lower Chesapeake Bay. Tagged tautog were released at the same sites where originally caught within 2 h of capture. Sites were continuously monitored with automated acoustic receivers between 9 November 1998 and 13 October 1999. Two sites consisted of natural bedform materials and two sites consisted of manmade materials. Ninety-four percent of tautog (n=15) released in fall 1998 remained inshore during winter at sustained water temperatures of 5–8°C, rather than moved offshore during winter as documented for tautog off New York, Rhode Island, and Massachusetts. Ninety-one percent (n=10) of tautog released in spring 1999 remained inshore during summer when water temperature was 27°C and in the absence of an important food item, blue mussels (*Mytilus edulis*). These findings conflict with assertions that tautog move to cooler water in summer when water temperatures reach 20°C. Tautog released at natural bedform sites were detected only at these sites throughout the study. Tautog released at manmade structures also displayed high site-utilization patterns, but several tautog periodically moved 2–10.2 km away from these sites over featureless bottom, a known deterrent to emigration for large temperate labrids in other waters. Benthic communities were similar at manmade sites and natural bedform sites, and movement away from manmade sites may have been influenced by habitat size as well as habitat structure. Understanding temporal and spatial utilization of habitats is an important first step to identifying essential fish habitat and to evaluating and protecting fishery resources within Chesapeake Bay and elsewhere.

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The labrid *Tautoga onitis* (tautog) is a highly prized game fish targeted by anglers fishing at natural and manmade structure (Briggs, 1977; Lucy and Barr, 1994). Tautog are distributed between Georgia (Parker, 1990) and Nova Scotia (Bigelow and Schroeder, 1953); peak abundance is found between Massachusetts and the Delaware Capes.[1] Slow growth rate, late age at maturity, predictable distribution, and localized population structure suggest high vulnerability to overexploitation (Hostetter and Munroe, 1993). Extended residence at accessible fishing sites may increase the potential for overexploitation; thus, residence and site-utilization patterns of tautog throughout this species' distribution range must be well understood for effective management of this resource.

Tag-recapture studies in New York, Rhode Island, and Massachusetts suggest that adult tautog spend spring and fall months inshore, may move offshore during the warmest summer months (Cooper, 1966; Briggs, 1969), and overwinter offshore (Cooper, 1966; Briggs, 1977). Tautog leave inshore waters at varying rates between July and October (Cooper, 1966) and are recaptured in coastal waters in fall (Cooper, 1966; Briggs, 1977), consistent with indirect observations on seasonal abundance (Stolgitis, 1970; Olla et al., 1974). In contrast, tag-recapture studies report limited evidence of a seasonal inshore-offshore migration for tautog in the Chesapeake Bay and coastal Virginia waters.[2] Seasonal abundance data also suggest that tautog remain inshore in Chesapeake Bay (Hostetter and Munroe, 1993) and in Delaware Bay (Eklund and Targett, 1991) during winter.

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[1] Atlantic States Marine Fisheries Commission (ASMFC). 1996. Fishery management plan for tautog, rep. 25, 56 p. [Available from ASMFC, 1444 Eye Street NW, Washington, DC 20005.]

[2] Virginia Game Fish Tagging Program. 1995–1999. Marine Resources Commission, 968 Oriole Dr. South, Suite 102, Virginia Beach, VA 23451.

The tag-recapture method is not a suitable method for evaluating site-utilization patterns because this technique does not provide information on the location of tagged animals between times of release and recapture. Furthermore, the tag-recapture method requires that tagged animals be recaptured, and be reported as recaptured, before any information is available. Ultrasonic telemetry, however, enables continuous observations on all tagged animals, in their natural environment, without requiring that tagged animals be recaptured (Winter, 1996). Previously, only in one other study (Olla et al., 1974) was ultrasonic telemetry used to monitor adult tautog. Olla et al. (1974) tagged and "tracked" 10 adult tautog ultrasonically in Great South Bay, NY, for up to 80 h after their release. Although an important study, their sample size was small and total observations too limited (<400 h, single season) to document seasonal occurrence and site-utilization patterns for this species.

Ultrasonic telemetry was selected to address seasonal occurrence and site-utilization patterns of adult tautog in lower Chesapeake Bay, given that tag-recapture methods can be applied only within limitations and given that the poor visibility and strong currents preclude direct underwater observations of this species in this turbid estuary. Rather than collect detailed positional data over short periods of time (days) for a few tautog, we chose to collect seasonal occurrence and site-utilization data for two large groups of tautog (n=16 and 11) at four specific sites located within known tautog habitat. Sites were monitored by using a fixed, submerged hydrophone array between November 1998 and September 1999. The first objective of our study was to determine if tautog remained inshore at natural and manmade structures in lower Chesapeake Bay during winter and summer. The second objective was to document and describe site-utilization patterns within inshore study sites. Data for daily activity patterns are presented elsewhere (Arendt et al., in press).

Materials and methods

Tautog were caught, tagged, and released at four sites situated within a 1.5 km × 6 km area near Cape Charles, Virginia (Fig. 1). Side-scan sonar (Sea Scan Technology, Ltd., White Marsh, VA) was used to measure dimensions of the four study sites and to map the surrounding seafloor. The Texeco Wreck, a 30 m × 100 m shipwreck, was located in 18 m of water west of the Susquehanna Channel (30–40 m deep) in an area characterized by flat, relatively featureless bottom topography (Wright et al., 1987). The three remaining sites (Airplane Wreck, Coral Lump, and Ridged Bottom) were located in 8–15 m of water east of the Susquehanna Channel in an area characterized by sand flats and deep, mud-bottomed channels (Wright et al., 1987). The Airplane Wreck (40 m × 20 m) consisted of concrete rubble. The Ridged Bottom (30 m × 100 m) and Coral Lump (100 m × 300 m) sites consisted of natural bedforms. Otter trawl, oyster dredge, and underwater video surveys indicated that all sites were densely populated by several species of sponges, colonial bryozoans, mollusks, and crustaceans.

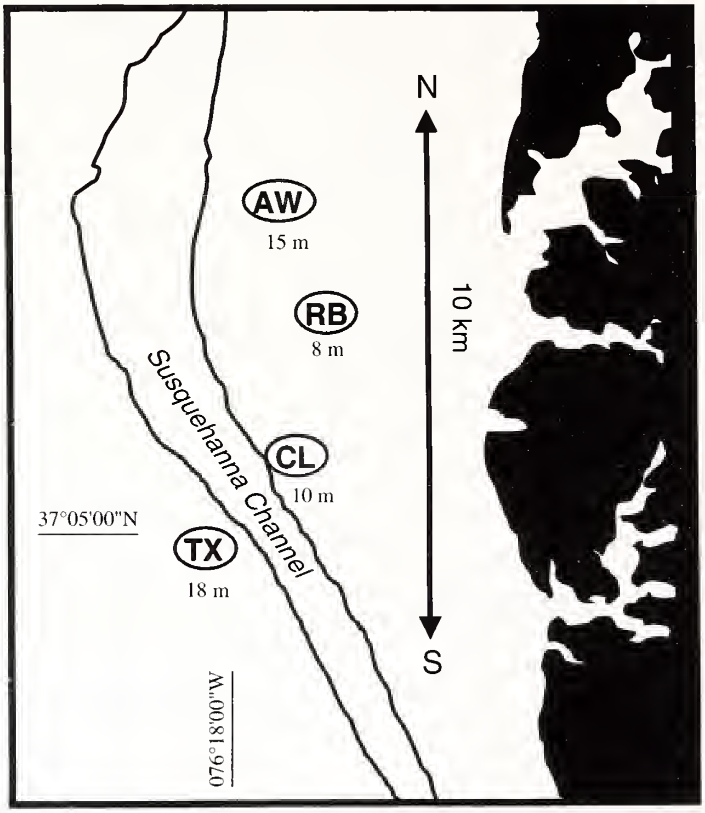

Figure 1

Location of study sites for telemetric study of tautog released in lower Chesapeake Bay near Cape Charles, VA. Texeco Wreck (TX) is located in 18 m of water on a plain west of Susquehanna Channel (30–40 m deep). Coral Lump (CL), Ridged Bottom (RB), and Airplane Wreck (AW) are located in 8–15 m of water on a flat east of the Susquehanna Channel.

Tautog were caught with standard two-hook bottom rigs baited with pieces of blue crab or clam and were brought aboard with a nylon landing net. Tautog were observed in an aerated live well up to 2 h before transmitters were implanted. Total length (mm) and sex (White, 1996) were recorded. Only tautog >400 mm TL were tagged ultrasonically. This minimum size increased the odds of transmitters weighing less than 1.25% of fish body weight in water (Winter, 1996), based on size-weight relationships for tautog in Virginia (Hostetter and Munroe, 1993; White, 1996). Tautog >400 mm were also reproductively mature (Hostetter and Munroe, 1993; White, 1996).

Surgical procedures were similar to those used in Nemetz and Macmillan (1988), Mortensen (1990), Holland et al. (1993), Szedlmayer (1997), and Thoreau and Baras (1997). In preparation for surgical implantation of transmitters, level-four anesthesia (Mattson and Ripple, 1989; Prince et al., 1995) was induced by immersing tautog in a 325-mg/L solution of MS-222. Once anesthetized, a small

(25-mm) incision was made on each fish immediately dorsal to the ventral midline between pelvic fins and anus with a sterilized, disposable razor blade. The transmitters was coated in sterile mineral oil and placed into the visceral cavities of tautog with the transducer end of the transmitter facing forward. Braided, polyglycolic acid sutures (Dexon®, I-III), surgical staples (Proximate Plus MD 35W®), and acrylic adhesive (Krazy glue®) were used to close the incision. Betadine was used periodically throughout the surgical procedure and antibiotics (Nu-Flor®) were injected intramuscularly to increase postsurgical survival (Schramm and Black, 1984; Bart and Dunham, 1990; Poppe et al., 1996). Tautog were revived in the aerated live well and released within 0.5 h after surgery. Preliminary evaluation of surgical procedures with "dummy" transmitters indicated 100% transmitter retention, 86% survival, and normal swimming, feeding, reproductive behavior, and physiology for tautog >400 mm TL held up to 418 days in captivity (Arendt, 1999). Tautog were fully recovered from surgery <1 to 6 days after release (Arendt, 1999) according to detection patterns recorded by automated acoustic receivers (Arendt and Lucy, 2000).

V-16-1H-R256 coded transmitters (16 mm × 48 mm, 9 g in water; Vemco, Ltd., Shad Bay, Nova Scotia, Canada) were used in our study. Signal repeat intervals for coded transmitters (69 kHz) varied randomly between 45 and 75 s, which extended battery life to 111 d. Transmitters were primarily detected with a submerged array of automated acoustic receivers (VR1, Vemco, Ltd.); however, transmitters were also detected from a research vessel with acoustic hydrophones (V10, VH65, Vemco, Ltd.) and an electronic receiver (VR60, Vemco, Ltd.).

Omnidirectional VR1 receivers were deployed 100–150 m to the west and east of the perimeter of each of the four sites. Detection radius for each receiver was approximately 400 m. Detection areas for both receivers were overlapped to create three distinct reception zones: a central reception zone common to both receivers and two peripheral reception zones unique to either receiver. VR1 receivers were moored 1.5–3.0 m above the seafloor to provide an unobstructed line-of-sight for transmitter signal reception (i.e. positioned above the "structure" associated with each site) and to reduce acoustic interference from suspended material associated with strong bottom currents. Mooring units consisted of a railroad wheel (227 kg), stainless steel aircraft cable (0.64 cm, 7 × 19 strand), and subsurface and surface floats. Receivers were retrieved every three to six weeks and detection data (transmitter ID, date and time of detection) were downloaded directly to a shipboard computer by means of a VR1-PC cable interface (Vemco, Ltd.).

Data for tautog released in fall 1998 were collected for the duration of the transmitter battery life. Data for tautog released in spring 1999 were collected until all VR1 receivers were permanently removed from each site. VR1 detections for each tautog were sorted into hourly bins and examined graphically. Because tautog are diurnally active and nocturnally quiescent (Olla et al., 1974; Arendt et al., in press), only daytime detection was used to determine site-utilization patterns. Tautog were considered resident at a particular site each day if they were detected at the same site throughout the day (morning, mid-day, evening). Total fish-days (sum of all days between date of first and last detection for all fish) for each calendar season were classified as 1) days when tautog were resident at the site of initial release, 2) days when tautog were detected at an alternative site, or 3) days when they were not detected at all. Site-utilization patterns of tautog were examined for each site in fall (from 9 Nov 98 to 20 Dec 98), winter (from 21 Dec 98 to 20 Mar 99), spring (from 21 Mar 99 to 20 Jun 99), and summer (from 21 Jun 99 to 9 Sep 99).

To increase the probability that tautog would be reported as recaptured should recapture occur, transmitters were labeled with the specimen's ID number, a $50 "reward" notice, and a phone number. Tautog were tagged externally with a small, orange t-bar anchor tag (TBA2; Hallprint, Holden Hill, South Australia) used by the Virginia Game Fish Tagging Program and with a larger, green t-bar tag (SHD-95; Floy Mfg., Seattle, WA) containing the specimen's ID number, a $50 reward" notice, and a phone number. Internal and external reward notices were included because Szedlymayer (1997) had observed that internal reward notices persisted longer than external reward notices for red snapper (*Lutjanus campechanus*) in the Gulf of Mexcio, and that internal reward notices were noticed accidentally. In addition to distinct marking of each tautog, colorful reward posters were posted at over 40 marinas, boat ramps, and tackle shops in lower Chesapeake Bay and literature describing the project was mailed to over 5000 homes and businesses.

Results

Twenty-seven adult tautog (400–514 mm TL) were tagged with ultrasonic transmitters and released (16 in fall 1998, 11 in spring 1999) near Cape Charles, VA (Table 1). Four tautog were released at each of the four sites in fall 1998 and at the Coral Lump and Texeco Wreck in spring 1999. Two tautog were released at the Ridged Bottom and one tautog was released at the Airplane Wreck in spring 1999. Similar numbers of tautog were tagged and released at manmade (n=13) and natural bedform (n=14) sites.

Eighty-one percent (n=22) of all tautog released were males; 19% were females. Thirteen percent (n=2) of tautog released in fall 1998 were female; 27% (n=3) of all tautog released in spring 1999 were female. Both female tautog released in fall 1998 (ID19, ID28) were released at the Texeco Wreck. One female tautog was released at the Texeco Wreck (ID37) and two female tautog were released at the Coral Lump (ID39, ID40) in spring 1999.

Ninety-four percent (n=15 of 16) of tautog released in fall 1998 remained inshore within lower Chesapeake Bay during winter. Ninety-one percent (n=10 of 11) of tautog released in spring 1999 remained inshore within the Bay during summer.

All tautog (n=14) released at natural sites remained inshore and were detected only at their respective release sites. Tautog released at the Ridged Bottom and Coral Lump sites were detected 99% of fish-days in fall, 71–91% of fish-days in winter, 64–100% of days in spring, and

Table 1

Summary of fish-days for data from 27 adult tautog (400–514 mm TL) tagged and released with ultrasonic transmitters at four sites in lower Chesapeake Bay near Cape Charles, Virginia. Recaptured tautog are noted with an asterisk (*). Abbreviations for sites: CL = Coral Lump; TX = Texeco Wreck; RB = Ridged Bottom; AW = Airplane Wreck.

					Fish days			
ID	Sex	TL	Site	Date released	At site	Not detected	At alternate site	Total
1	M	432	CL	9 Nov 1998	174	8	0	182
18	M	406	CL	9 Nov 1998	155	19	0	174
19	F	495	TX	10 Nov 1998	58	105	2	165
20*	M	470	TX	10 Nov 1998	0	167	1	168
21	M	406	RB	10 Nov 1998	88	10	0	98
22	M	400	RB	10 Nov 1998	178	0	0	178
23	M	483	AW	13 Nov 1998	152	13	0	165
24	M	432	AW	13 Nov 1998	112	45	0	157
25	M	432	CL	3 Dec 1998	183	3	0	186
26	M	400	CL	3 Dec 1998	177	4	0	181
27	M	514	TX	4 Dec 1998	177	0	0	177
28	F	413	TX	4 Dec 1998	42	130	13	185
29*	M	400	AW	7 Dec 1998	147	14	1	162
30	M	419	AW	7 Dec 1998	46	21	0	67
31	M	445	RB	8 Dec 1998	144	24	0	168
32	M	419	RB	8 Dec 1998	88	39	0	127
33	M	406	TX	21 Apr 1999	0	136	6	141
34*	M	432	CL	28 May 1999	69	0	0	69
35	M	445	TX	28 May 1999	97	7	0	104
36	M	—	TX	28 May 1999	103	1	0	104
37*	F	445	TX	28 May 1999	99	5	0	104
38*	M	483	CL	7 Jun 1999	59	0	0	59
39*	F	483	CL	7 Jun 1999	59	0	0	59
40*	F	432	CL	7 Jun 1999	59	0	0	59
41	M	445	AW	7 Jun 1999	10	2	2	14
42*	M	406	RB	9 Jun 1999	58	0	0	58
43	M	406	RB	9 Jun 1999	56	2	0	58

98–100% of fish-days in summer (Fig. 2). Reduced detection in winter and spring was partially attributed to two tautog (ID21, ID32) that were detected 46 d and 75 d less than the mean (175 d) for other tautog (n=12) released at the same time. To provide a more conservative estimate of site utilization, these tautog were listed as "not detected" at these sites for these days.

Eighty-five percent (n=11) of tautog released at man-made structures remained inshore and all but three of these were detected only at their respective release sites. Site utilization by tautog at the Texeco Wreck was low (34–71% of fish-days) in all seasons (Fig. 2). One tautog (ID20) released at the Texeco Wreck was only detected at the Texeco Wreck for three hours after being tagged and released in fall 1998. Two additional tautog (ID19, ID28) spent 64–70% of fish-days away from the Texeco Wreck in fall, winter, and spring, and a fourth tautog (ID33) spent 100% of fish-days away from the Texeco Wreck in spring and summer. One of these tautog (ID28) was detected at the Coral Lump for 6 days in January 1999 (VR1 receivers) and all three tautog were regularly detected with the VR60 receiver at a site 2 km south of the Texeco Wreck. Tautog released at the Airplane Wreck were resident 99% of fish-days in fall, 66% of fish-days in winter, and 58% of fish-days in spring (Fig. 2). Reduced detection in winter resulted partially from one tautog (ID30) being detected 108 days less than the mean (175 days) for other tautog (n=12) released at the same time. To provide a more conservative estimate of site utilization, this tautog was listed as "not detected" at the Airplane Wreck for these days. No summer data were available for tautog at the Airplane Wreck because the single tautog (ID41) released there in spring 1999 was detected at this site for two days following release, was later detected at the Texeco Wreck, and then was never detected again at any site. Tautog 41 was listed as "not detected" at the Airplane Wreck from the time of

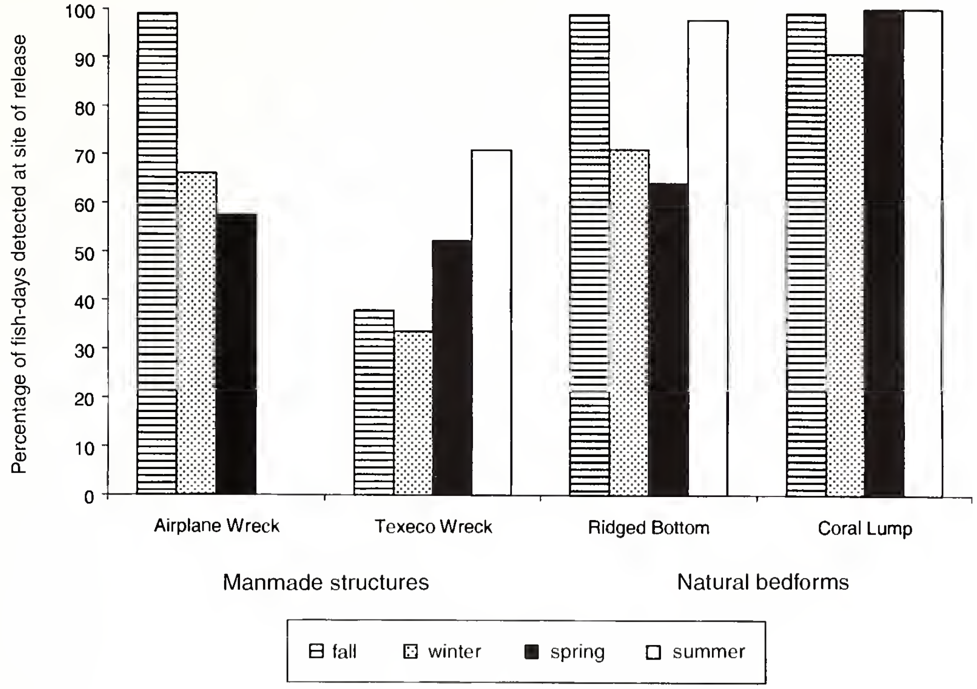

Figure 2

Seasonal site-utilization patterns of tautog released at manmade (Airplane Wreck and Texeco Wreck) and natural bedform (Ridged Bottom and Coral Lump) sites near Cape Charles, Virginia, in fall (9 Nov 98 to 20 Dec 98), winter (21 Dec 98 to 20 Mar 99), spring (21 Mar 99 to 20 Jun 99), and summer (21 Jun 99 to 9 Sep 99). Site-utilization patterns were greatest for tautog released at natural bedform sites.

leaving this site until VR1 receivers were removed from this site, 112 d later.

Eight tautog (27%) released in our study were subsequently recaptured (Table 1). Two tautog (ID20, ID29) released in fall 1998 were recaptured (13%) away from release sites by commercial fishermen in spring 1999. Tautog 20 was released at the Texeco Wreck on 10 November 1998 and either left this site the same day or its transmitter failed to transmit data. This tautog was subsequently recaptured 10.2 km northeast of the Texeco Wreck in a crab pot on 27 April 1999, 169 days later. Tautog 29 was first caught at the Airplane Wreck on 13 November 1998 and held in a wire cage with several other tautog at the Airplane Wreck for five days as part of a catch-release mortality study on tautog.[3] After being released at the Airplane Wreck on 18 November 1998, this tautog was recaptured at the Airplane Wreck on 7 December 1998 (19 d later) and ultrasonically tagged and released. Tautog 29 remained at the Airplane Wreck until 12 May 1999, then was recaptured in a gill net 2 km east of the Airplane Wreck on 19 May 1999. Recreational fishermen recaptured six tautog released in spring 1999 (55%) at the same sites where these fish were released 114–211 d earlier and 8–13 weeks after VR1 receivers were removed from sites.

Discussion

Tautog remained inshore within lower Chesapeake Bay during winter, at sustained water temperatures of 5–8°C (Arendt et al., in press). Although detected at these water temperatures, tautog overall were detected less than during other times of the year, most likely because tautog remained inactive and within structures for several days at a time (Arendt et al., in press). Inshore occurrence of tautog in winter has been observed in eastern Long Island Sound,[4] Delaware Bay (Eklund and Targett, 1991), and lower Chesapeake Bay (Hostetter and Munroe, 1993). When water temperatures remains above 9–10°C,[5] a viable winter inshore fishery exists for tautog within lower Chesapeake Bay. Within the winter fishery, inshore catches occur predominantly in December and March, whereas offshore catches occur predominantly in January and February. Occurrence of an inshore winter fishery for tautog in Virginia is unique within this species' geographic distribution.

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[3] Lucy, J. A., and M. D. Arendt. 1999. Exploratory field evaluation of hook-release mortality in tautog (*Tautoga onitis*) in Lower Chesapeake Bay, Virginia. Rep. VMRC-99-10, 11 p. [Available from Marine Advisory Program, Virginia Institute of Marine Science, Gloucester Point, VA 23062.]

[4] Auster, P. J. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North and Mid-Atlantic)—tautog and cunner. U.S. Fish and Wildlife Service Biological Report 82 (11.105). U.S. Army Corps of Engineers Rep. TR EL-82-4, 13 p. NOAA's National Undersea Research Program, Univ. Connecticut at Avery Point, Groton, CT 06430.

Tautog remained inshore during the summer at a maximum sustained water temperature of 27°C (Arendt et al., in press). Summer residence data have been supported by direct underwater observations.[6] Infrequent recreational catches of tautog in lower Chesapeake Bay during summer have also been reported.[2] Inshore, summer residence of tautog has been documented for Great South Bay, NY, when water temperatures were 19–24°C (Olla et al., 1974) and Narragansett Bay, RI, at maximum sustained water temperatures of 22°C.[7] These findings contradict reports from Virginia (Adams, 1993), New York (Briggs, 1969), and Rhode Island (Cooper, 1966) that adult tautog may move offshore to cooler water during summer.

Tautog remained inshore during summer in the absence of blue mussels (*Mytilus edulis*), a primary food item of tautog in northern areas (Bigelow and Schroeder, 1953; Olla et al., 1974). In June 1998–99, using underwater video, otter trawl, and oyster dredge, we documented large clusters of live blue mussels at study sites and noted growth of mussels on VR1 mooring units. By July 1998–99, blue mussels were not present at any of these sites. Absence of mussels in July in both years was most likely due to lethal effects of water temperatures ≥27°C (Wells and Gray, 1960).

Because blue mussels are not present in lower Chesapeake Bay year round, the diet of tautog inhabiting lower Chesapeake Bay throughout the year may be more diverse than that of tautog in northern areas. Stomach contents from an ultrasonically tagged tautog recaptured in October 1999 at the Ridged Bottom site consisted primarily of the bryozoan *Alcyinidium verilli*. At an artificial fishing reef near Cape Charles, VA, tautog consumed a variety of crustaceans, shellfish, bryozoans, and hydroids.[8] Similar temporal distributions of blue mussels have been reported in Delaware Bay, where diets of tautog subsequently shift towards alternative, less nutritious food items at times when blue mussels are unavailable.[9]

Year-round occurrence of adult tautog in Chesapeake Bay differs from seasonal (spring and fall) inshore occurrence of adult tautog in Great South Bay, NY (Olla et al., 1974; Briggs, 1977), Narragansett Bay, RI (Cooper, 1966), and the Weweantic River Estuary, MA (Stolgitis, 1970). Year-round occurrence of ultrasonically tagged tautog was consistent with large-scale patterns of occurrence of tautog from conventionally tagged tautog (127–584 mm TL) in the Virginia Game Fish Tagging Program between 1995 and 1999.[2,5] Of 563 recaptured tautog that were originally tagged in lower Chesapeake Bay, excluding Cape Charles, and adjacent coastal waters, 85% (n=476) were recaptured at the same sites where released 0–1214 d earlier, including 20 multiple recaptures of individuals at the same sites where originally released. High incidence of recaptures of tautog at the same sites where they were released occurred during all seasons. Only 5% of all recapture events involved movement (8–97 km) of tautog between Chesapeake Bay and adjacent coastal waters.

Daily detection patterns were almost always similar for both VR1 receivers at sites, indicating that tautog remained within the central signal reception area of both VR1 receivers and in the general vicinity of sites throughout the entire day. Tautog were generally not detected by VR1 receivers at night; however, tautog likely remained at sites throughout the night (Arendt et al., in press). Tautog were likely detected less often (or not at all) at night because of nocturnal quiescence in or near structure (Olla et al., 1974) and therefore were effectively out of range of VR1 receivers because of the presence of an acoustic barrier (Matthews, 1992; Pearcy, 1992; Bradbury et al., 1995,1997; Zeller, 1997).

Featureless bottom topography, a known deterrent to emigration for large, temperate labrids (*Notolabrus tetricus, N. fucicola, Pictilabrus laticlavius, Pseudolabrus psittaculus)* in Tasmania (Barrett, 1995), did not act as a deterrent to emigration for tautog in lower Chesapeake Bay. Two tautog (ID20, ID28) released at the Texeco Wreck in fall 1998 traversed a wide (2-km), deep (37–40 m) mud-bottomed channel (Wright et al., 1987) on at least three occasions, and one of these tautog (ID20) was subsequently recaptured. Two tautog (ID19, ID28) traveled between the Texeco Wreck and a site 2 km south of the Texeco Wreck on at least 12 occasions. A third tautog (ID33) left the Texeco Wreck almost immediately after being released and was subsequently detected (VR60 receiver) at this site throughout the remainder of our study.

Movement by tautog was assumed to represent actual movement by tagged tautog as opposed to movement of a predator that may have preyed upon tagged tautog, even though these three tautog were never recaptured nor visually observed after release. Adult tautog are very infrequently preyed upon by sharks in Virginia (Gelsleichter et al., 1999); however, sharks are not likely present in Chesapeake Bay when water temperatures are 5–8°C. At these water temperatures, large striped bass (*Morone saxatilis*) pose the only possible predatory threat to tautog. A recent study on feeding habits of adult striped bass in Chesapeake Bay found no tautog in the stomachs of more than 2000 striped bass, many of which were collected from the Chesapeake Bay Bridge-Tunnel complex, a well-known fishing area for adult tautog (Walters, 1999).

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[5] White, G. G., J. E. Kirkley, and J. A. Lucy. 1997. Quantitative assessment of fishing mortality for tautog (*Tautoga onitis*) in Virginia. Preliminary report, 54 p. Department of Fisheries Science and Marine Advisory Program, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

[6] Hager, C. 1999 (July). Personal communication of direct underwater observation of tautog at Plantation Light (3–8 m depth), 2 km southeast of Texeco Wreck study site. School of Marine Science, College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

[7] Castro, K. 1999 (October). Personal communication of water temperature observation for Narragansett Bay. East Farm–Fisheries Center, Univ. Rhode Island, Kingston, RI 02881.

[8] Feigenbaum, D., C. Blair, and A. J. Provenzano. 1985. Artificial reef study—year II report. Virginia Marine Resources Commission rep. VMRC-83-1185-616, 57 p. VA Institute of Marine Science, Gloucester Point, VA 23062.

[9] Steimle, F., K. Foster, W. Muir, and B. Conlin. 1999. The diet of tautog collected on an artificial reef in Delaware Bay and interannual effects of prey availability (and notes on other tautog diet studies in the middle Atlantic Bight). First Biennial Conference on the biology of tautog and cunner, Mystic, CT, 30 November–1 December 1999. National Marine Fisheries Service, Highlands, NJ 07732.

All tautog detected or recaptured away from release sites were released at manmade sites, which also happened to be the smallest sites. No information was available regarding the origin of these manmade sites, but both have been in place for at least 20 years.[10,11] Stone et al. (1979) concluded that artificial reefs reach a stable state after five years. Benthic macrofauna collected at manmade sites during our study were similar to benthic macrofauna collected at natural sites, suggesting that food may have been similar between manmade and natural sites. Given these observations, habitat size may be an important factor for adult tautog in determining the scale of local movements between adjacent habitats. Understanding the relationship between habitat size and site utilization warrants further investigation, especially with recent increased interest in the construction of artificial habitats for purposes of stock enhancement and enhanced fishing opportunities for tautog.

Sex ratio of female to male tautog in our study was heavily skewed (1:3.5) towards male tautog due to opportunistic sampling, and the preponderance of male tautog likely contributed to the high levels of site utilization observed in our study. In laboratory settings, adult male tautog aggressively defend territories throughout most of the year (Olla et al., 1978, 1980) and only during the spawning season are female tautog permitted to enter territories (Olla and Samet, 1977; Olla et al., 1981). Overall activity, including male agonistic behavior, also decreases as water temperatures approach annual minimum and maximum values (Olla et al., 1978, 1980). Although sample size in our study was too small to distinguish site-utilization patterns by sex, it is worth noting that both tautog that left the Texeco Wreck in Nov–Dec and that periodically returned to this site throughout the winter and spring were females. In contrast, during the spring spawning season, three females (one at Texeco Wreck, two at Coral Lump) remained at release sites throughout the spring–summer monitoring period and all three were subsequently recaptured by recreational fishermen at these same sites in the fall. More sex-specific data are needed to fully comprehend what role reproductive biology and social structure have on seasonal site-utilization patterns.

Site-utilization patterns exhibited by ultrasonically tagged tautog were consistent with patterns reported for tautog released at these same sites from the Virginia Game Fish Tagging Program (VGFTP). Between April 1998 and October 1999, 40 tautog, tagged and released at these sites, were recaptured, including one tautog recaptured twice at the same site. Six of eight (75%) tautog originally released at the Texeco and Airplane Wrecks were recaptured away from these sites. Of these six tautog, three moved to the Coral Lump and Ridged Bottom and three moved to sites located 26.9 to 43.2 km away in lower Chesapeake Bay. In contrast, 32 tautog tagged and released at the Coral Lump and Ridged Bottom sites were recaptured, all but two (which moved from the Ridged Bottom to the Coral Lump) were recaptured where released. One additional fish moved to the Coral Lump from an artificial reef located 4 km to the northeast and within 2 km of where both tautog were recaptured by commercial fishermen in spring 1999.

Ultrasonically and conventionally tagged tautog released near Cape Charles, VA, in lower Chesapeake Bay demonstrated high site utilization at and high site affinity (returned to release sites after short emigration) for release sites. Extended residence by tautog at familiar sites during annual environmental extremes is considered more beneficial than emigration to more optimal environmental conditions because residence at familiar sites reduces the risk of not finding suitable shelter, food, mates, or of encountering predators (Olla et al., 1978). Although directed seasonal offshore movements were not observed in our study, movements between adjacent inshore locations occurred several times, including movement to adjacent locations during the periods of seasonal thermal extremes. Understanding temporal and spatial utilization of habitats is an important first step to identifying essential fish habitat and critical to evaluating and protecting fishery resources within Chesapeake Bay and elsewhere.

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Abstract—Mitochondrial DNA (mtDNA) haplotypes of coho salmon (*Oncorhynchus kisutch*) sampled from northern Pacific Ocean and Bering Sea drainages formed two monophyletic clades between which nucleotide divergences averaged 2.95 substitutions per 1000 nucleotides. These data were obtained from restriction endonuclease digestions of PCR products that included over 97% of the mtDNA genome and resolved 16 different haplotypes in 258 fish from 13 locations. Comparisons of haplotype compositions of populations indicated that the Bering Sea drainages and one Kodiak Island population clustered separately from nine other Gulf of Alaska populations, including one from Asia. Rates of gene flow among populations estimated from haplotype frequencies (assuming an equilibrium between gene flow and random drift) were low (about one female per generation between drainages within regions) in relation to allozyme-based estimates of gene flow for other Pacific salmon species. Much of the haplotype frequency variation was within-region variation. Haplotypes from both clades occur in many extant populations, suggesting that gene flow, population movements, or recolonization followed divergence of refugial isolates. Nested clade analysis of the geographic distribution of mtDNA haplotypes indicated that coho salmon demographic history has been influenced by recent isolation by distance and that historic population fragmentation was preceded by range expansion. These observations are consistent with effects expected from Pleistocene glacial advances and retreats.

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Phylogeographic analysis of mitochondrial DNA variation in Alaskan coho salmon, *Oncorhynchus kisutch*

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In drainages flowing into the Gulf of Alaska and Bering Sea, coho salmon (*Oncorhynchus kisutch*) are the least numerous and their population structure the least understood of Pacific salmon species (*Oncorhynchus* spp.). Many populations spawn in late fall or winter in remote drainages that are difficult to access. Spawning populations are often small and separated widely (Sandercock, 1991). In larger rivers spawning adults may return to small, often transient, headwater streams. After emergence, fry and juveniles may move to rearing areas, usually much lower in the river (Sandercock, 1991), forming complex admixtures from spawning populations in large drainages.

Studies of allozyme variation have provided insight into the population structure of Pacific salmon species (e.g. Zhivotovsky et al., 1994, and references therein). Patterns of genetic variability often provide evidence of relationships between populations resulting from coancestry or gene flow, and genetic divergence among populations may be used for stock identification (Shaklee et al., 1999). In coho salmon, the low level of allozyme variation resolves relatively little population genetic structure (Reisenbichler and Phelps, 1987; Wehrhahn and Powell, 1987; Bartley et al., 1992; Pustavoit, 1995). This low level of allozyme variation is consistent with numerous spawning populations that have small effective sizes and low levels of gene flow, such as that of coho salmon.

Analysis of DNA variation adds dimensions of interpretation not possible with allozyme data (Avise et al., 1988; Avise, 1989). "Gene trees" for mitochondrial DNA (mtDNA) haplotypes are especially informative. The mitochondrial genome is transmitted (primarily) maternally (Gyllensten et al., 1991), and mtDNA is haploid and clonally inherited with no recombination. Consequently, mutations accumulate over time within a clonal mtDNA line or haplotype. Comparison of different haplotypes provides a basis for reconstruction of matriarchal genealogies. There is also evidence that mtDNA sequences may diverge (evolve) faster than many nuclear sequences (Brown et al., 1982). The extent of nucleotide divergence provides a temporal basis for comparing haplotype lineages. The rates of divergence of mtDNA have been roughly estimated for a number of species pairs by comparing observed nucleotide sequence divergence with fossil records that document the emergence of those species (Brown et al., 1979; Shields and Wilson, 1987 and references therein). Although applications of these molecular "clocks" are questionable when extended to species for which the fossil record is poor or missing, deductions about relative (as opposed to absolute) divergence times can be made from the extent of nucleotide change within a species. Furthermore, the geographic distri-

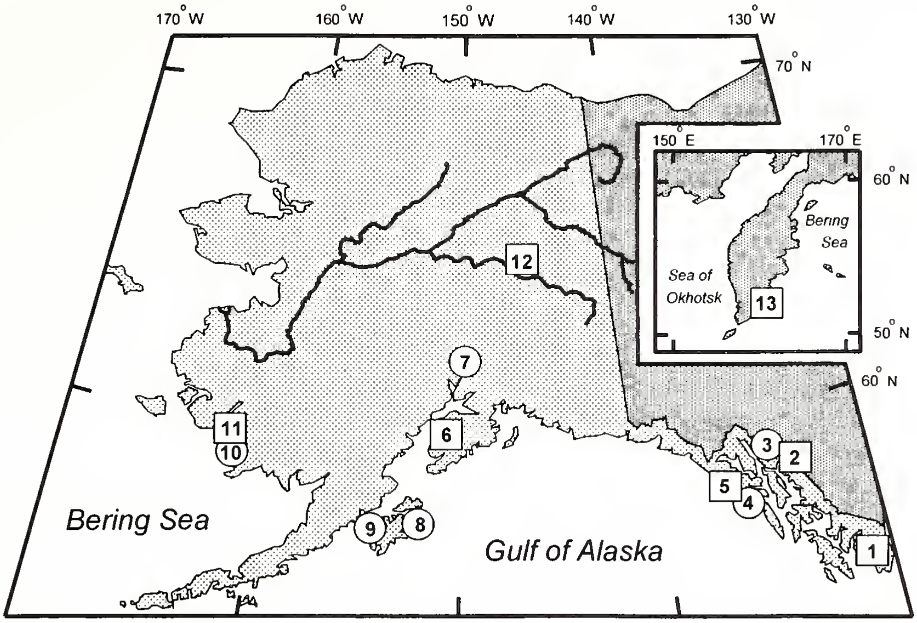

Figure 1

Sites from which coho salmon were sampled for mtDNA analysis. Squares denote samples used in both the preliminary and secondary analyses; circles denote collections used only for the secondary analysis. Locations are Hugh Smith River (1), Fish Creek, a Taku River tributary (2), Berner's River (3), Indian River (4), Ford Arm River (5), Crooked Creek (6), Little Susitna River (7), Buskin River (8), Karluk River (9), Eek River (10), Kanektok River (11), Delta Clearwater River, a Yukon River tributary (12), and Kamchatka River (13).

bution of haplotypes and their genealogical relationships can provide information about the historic demography and gene flow of a species. Templeton and colleagues (e.g. Templeton and Sing, 1993, Castelloe and Templeton, 1994; Templeton, 1998) have developed methods to examine both the shape of the "gene tree" and the geographic distribution of haplotypes, which they term "nested clade analysis of geographic distances."

The objectives of our study were to survey the geographic distribution of mtDNA variation in Alaskan coho salmon populations along the Gulf of Alaska and Bering Sea and to use that information and the mtDNA haplotype "gene tree" to deduce the nature of the historic demographic processes that influenced the contemporary geographic distribution of coho salmon.

Materials and methods

Coho salmon were sampled from 12 drainages in Alaska and one in Asia (Fig. 1). Samples of heart tissue from each specimen were preserved in 95% ethanol or a solution of 20% dimethyl sulfoxide (DMSO) and 0.25M ethylenediaminetetraacetic acid (EDTA) at pH 8, saturated with NaCl (Seutin et al., 1991).

Total genomic DNA was isolated by phenol-chloroform extraction (Wallace, 1987) or with Puregene DNA^{TM} isolation kits (Gentra Systems Inc., Minneapolis, MN). Sequences were PCR-amplified using primers that targeted seven regions of the mtDNA genome in pieces that range from about 2115 to 2689 base pairs (bp) (Fig. 2, Table 1). The regions were designated ND3/ND4 (including genes for the NADH dehydrogenase-3 subunit and NADH dehydrogenase-4L and -4 subunit genes), ND5/ND6 (including genes for the NADH dehydrogenase-5 and -6 subunits), Cytb/D-loop (including the cytochrome *b* gene and the control region), 12S/16S (including 12S rRNA gene and most of the 16S rRNA gene), ND1/ND2 (including the NADH dehydrogenase-1 and NADH dehydrogenase-2 subunit genes), COI/COII (including most of the cytochrome oxidase I subunit gene and the cytochrome oxidase II subunit gene), and A8/COIII (including genes for the ATPase-8 and -6 subunits and the cytochrome oxidase III subunit gene). The seven mtDNA regions were amplified by denaturation at 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C [0.2 mM of each dNTP, 0.2μ M of each primer, 2 mM MgCl$_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.3 with 1 unit of Taq polymerase (Perkin Elmer, Norwalk, CT) in a 50-μL reaction], except that amplifications of regions A8/COIII and ND3/ND4 required 3 mM instead of 2 mM MgCl$_2$.

Subsamples of PCR products of each mtDNA region were digested with each of 12 restriction enzymes. The endonucleases recognized six bases (*Ase* I), multiple six-base sites (*Ava* I, *Hind* II, *Sty* I), multiple 5 base sites (*Bst*N I), and four bases (*Bst*U I, *Cfo* I, *Dde* I, *Hinf* I, *Mbo* I, *Msp* I, *Rsa* I). Digestion reactions were carried out under conditions recommended by the manufacturers. The resulting frag-

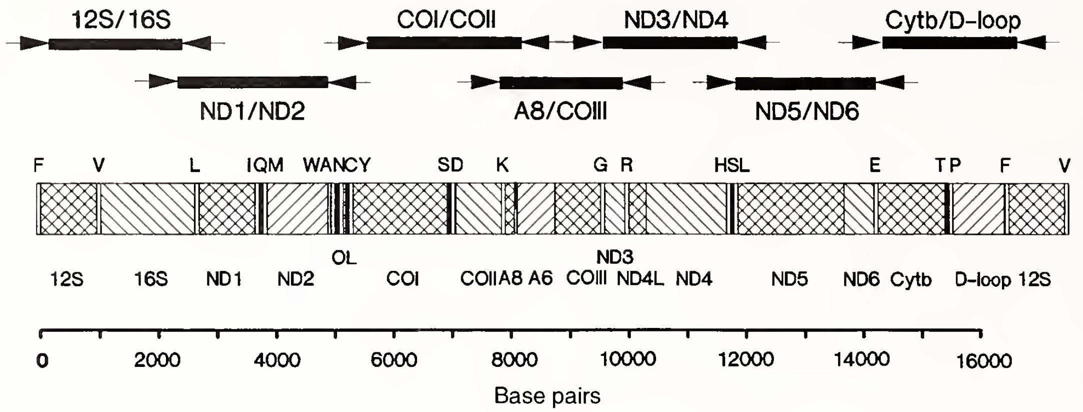
12S/16S
COI/COII
ND3/ND4
Cytb/D-loop
ND1/ND2
A8/COIII
ND5/ND6
F V L IQM WANCY SD K G R HSL E TP F V
OL
ND3
12S 16S ND1 ND2 COI COII A8 A6 COIII ND4L ND4 ND5 ND6 Cytb D-loop 12S
0 2000 4000 6000 8000 10000 12000 14000 16000
Base pairs

Figure 2

The gene map of linearized mtDNA and fragments that can be PCR amplified in coho salmon. The map is based on the sequence of *O. mykiss* mtDNA (Zardoya et al., 1995). More precise details of the range spanned by each primer pair are provided in Table 1.

Table 1

Primers for PCR amplification of coho salmon mitochondrial DNA (mtDNA), location in relation to *O. mykiss* (Zardoya et al., 1995), PCR fragment sizes, and fragment overlaps (bp).

Region	Sequence	*O. mykiss* locations	Fragment size	Overlap	Source
12S/16S	5′ AATTCAGCAGTGATAAACATT 3′	1234–1254			1
	5′ AGATAGAAACTGACCTGGATT 3′	3615–3635	2402		1
				121	
ND1/ND2	5′ ACCTCGATGTTGGATCAGG 3′	3515–3533			1
	5′ ATTAAAGTGNTTGA(T/G)TTGCATTC 3′	6181–6203	2689		1, 5
				–430	
COI/COII	5′TAATCGTCACAGCCCATGCCTTCGT 3′	6634–6658			2
	5′ GGTCAGTTTCAGGGTTCAGGTTTAGC 3′	9079–9104	2471		2
				166	
A8/COIII	5′ CTAGTGACATGCCCCAACTCAACC 3′	8939–8962			2, 3
	5′ TCATAAGGCGGTCATGGACTTAAACC 3′	11028–11053	2115		2, 3
				480	
ND3/ND4	5′ TTACGCGTATAAGTGACTTCCAA 3′	10574–10596			2, 3
	5′ TTTTGGTTCCTAAGACCAATGGAT 3′	12881–12904	2331		2, 3
				32	
ND5/ND6	5′ AACAGCTCATCCATTGGTCTTAGG 3′	12873–12896			2, 4, 5
	5′ TTACAACGATGGTTTTTCATGTCA 3′	15319–15342	2470		2, 4, 5
				19	
Cytb/D-loop	5′ TGAA(G/A)ACCACCGTTGTTATTCAA 3′	15324–15347			2, 4, 5
	5′ TAGGGCCTCTCGTATAACCG 3′	1321–1340	2659		2, 4, 5
				107	
12S/16S					

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[1] Consensus from Anderson et al. (1981); Anderson et al. (1982); Bibb et al. (1981); Roe et al. (1985); Chang et al. (1994).

[2] Unpublished mtDNA sequence data from our lab.

[3] Thomas and Beckenbach (1989).

[4] Cronin et al. (1993).

[5] Carney et al. (1997).

ments were separated by electrophoresis through a 1.5% agarose gel (a mixture composed of one part Ultra Pure^{TM} agarose [BRL Gibco, Grand, NY] and two parts Synergel^{TM} [Diversified Biotech Inc., Boston, MA]) in 0.5×TBE buffer (TBE is 90 mM Tris-boric acid, and 2 mM EDTA, pH 7.5). DNA in the gel was stained with ethidium bromide and photographed on an ultraviolet light transilluminator. Digests that produced fragments too small for detection in agarose/Synergel^{TM} gels were resolved in 12% polyacrylamide (29:1 acrylamide:bisacrylamide; 1×TBE) gels. DNA fragments separated in polyacrylamide gels were stained with SYBR Green 1 Nucleic Acid Stain^{TM} (Molecular probes, Eugene, OR), which is more sensitive than ethidium bromide. Either a 1-kilobase (kb) ladder or *Hae* III digested $\phi\chi^{174}$ RF phage DNA (BRL Gibco, Grand, NY) was used as a molecular weight reference for estimating restriction fragment sizes.

Restriction sites were inferred from fragment patterns that could be related to each other by the gain or loss of a single site. Composite haplotypes were constructed from restriction fragment patterns of all restriction enzymes across all mtDNA PCR regions. Using the rules of Castelloe and Templeton (1994) to resolve ambiguities, we constructed the single most probable parsimonious tree depicting restriction site changes between haplotypes. Using REAP (McElroy et al., 1990), we estimated haplotype (nucleon) and nucleotide diversities within populations (Nei, 1987) as well as average nucleotide divergences between populations. Nucleotide divergence between populations takes into account both the haplotype frequency differences between populations and the nucleotide divergences between haplotypes (Nei and Tajima, 1983; Nei, 1987; Nei and Miller, 1990). Homogeneity of haplotype diversities among populations was tested by using the Monte-Carlo simulation in REAP (McElroy et al., 1990)(10,000 iterations; Hedges, 1992) to establish probability levels for goodness-of-fit statistics (Roff and Bentzen, 1989).

Populations were clustered from pair-wise nucleotide divergences by using the Fitch and Margoliash (1967) least-squares method (FITCH in PHYLIP; Felsenstein, 1995). For comparisons between populations, the precision of estimates of nucleotide divergence depends on sample size. Therefore, stability of the topology was examined by bootstrapping (2000 iterations; Hedges, 1992) over individuals within each collection. A consensus tree (CONSENSE in PHYLIP; Felsenstein, 1995) that shows the stability of the topology, but not the branch lengths, was generated from the set of bootstrapped trees.

The hierarchical structure of the expanded set of coho samples was analyzed by analysis of molecular variance (AMOVA; Excoffier et al., 1992) with Arlequin (Schneider et al., 1997). Collections were grouped geographically into four regions: Southeast Alaska, Southcentral Alaska, Bering Sea, and Asia. With appropriate choices of divergence matrices, the analysis can examine the structure from haplotype frequencies (e.g. Weir, 1996) or from nucleotide diversities based on paths between haplotypes traced through a haplotype tree (Excoffier et al., 1992). Significance (P_{MC}) of Φ-statistics (Excoffier et al., 1992) was estimated from distributions of the statistics generated by 17,000 permutations (Hedges, 1992) at the appropriate level of hierarchy.

Nested clade analysis of geographical distributions of haplotypes and subclades (e.g. Templeton and Sing, 1993; Castelloe and Templeton, 1994; Templeton, 1998) was conducted with GEODIS 2.0 (Posada et al., 2000).

Results

Our general approach was to conduct a broad preliminary survey to obtain genome-wide information for mtDNA restriction site variation. Subsequently, we focused on the variable restriction sites and examined larger sample sizes and additional populations. From those results we constructed a fine-scale mtDNA "gene tree" and analyzed the geographic distributions of mtDNA lineages to deduce the nature of the historical demographic changes that influenced present-day population structure. The approach also allowed us to determine the effects on estimates of molecular parameters that occur when analyses focus on variable restriction sites.

Diversities of coho salmon mtDNA

Ten coho salmon from each of seven drainages (Fig. 1) were analyzed to survey broadly the species' mtDNA variability using 12 restriction endonucleases (Appendix 1). The total number of restriction sites inferred from restriction fragment patterns was 298 (an average of 291.28 per haplotype), which corresponds to 1284 nucleotides (an average of 1254.80), or a maximum of 7.73% (an average of 7.56%) of the coho salmon mtDNA genome (Table 2). Sixteen sites (1.2% of the total) were variable. Individually, the regions averaged between 29 and 57 restriction sites, which correspond to a maximum of between 5.58% and 9.57% of the nucleotides in a region. Although the amplified regions had some overlaps (Table 1), no variable sites were observed in the areas of overlap; and no invariant sites were shared between regions, except possibly *Dde* I sites in the 408-bp overlap between A8/COIII and ND3/ND4 (Table 1). Because of the large total number of sites examined, a few overlapping *Dde* I sites would cause only a slight decrease in nucleotide diversity estimates and have little effect on nucleotide divergence estimates.

Restriction site variation was observed in five of the seven PCR-amplified mtDNA regions for the 12 restriction endonucleases used. Between zero and five variable sites were observed per region. No variation was detected in the A8/COIII and ND3/ND4 regions. The largest number of variable sites (5) and the highest level of nucleotide diversity (5.99 substitutions per 1000 base pairs) were observed in the ND5/ND6 region (Table 2).

Because there is no recombination between heterologous mtDNA molecules, the composite haplotype is the appropriate unit to consider in genetic analysis (e.g. Avise, 1989). Our preliminary survey discovered 11 haplotypes (Table 3). As a whole, the sample of 70 fish had a haplotype diversity of 0.803 and a nucleotide diversity of 1.70 substitutions per 1000 base pairs. Haplotype diversities within

Table 2

Number and variability of restriction sites observed in each of the seven mtDNA regions we examined using 12 restriction endonucleases in our preliminary survey.

Region	Fragment size	Mean number of sites	Mean number of nucleotides	% coverage	Number of variable sites	Number of haplotypes	Haplotype diversity (±SE)	Nucleotide diversity (per 1000)
12S/16S	2402	53.5	226.7	9.44	3	4	0.485 ±0.042	2.15
ND1/ND2	2689	41.5	184.7	6.87	3	4	0.485 ±0.042	2.65
COI/COII	2471	39.7	168	6.8	3	3	0.057 ±0.038	0.25
A8/COIII	2115	29	126.7	5.99	0	1	0	0
ND3/ND4	2331	31	130	5.58	0	1	0	0
ND5/ND6	2470	36.5	157.2	6.36	5	4	0.470 ±0.040	5.99
Cytb/D-loop	2659	57	248.7	9.35	2	3	0.670 ±0.014	1.87
Total	16,642	291.28	1254.8	7.54	16	11	0.803 ±0.024	1.70

Table 3

Observed numbers of each mtDNA haplotype, haplotype diversity, and nucleotide diversity (substitutions per 1000 bp) within collections of coho salmon examined in a preliminary survey of North Pacific Ocean coho salmon (Nei and Tajima, 1983; Nei ,1987). Standard errors are in parentheses. Homogeneity of haplotype frequencies ($P_{MC} < 10^{-4}$) was tested with using Monte-Carlo simulation based on 10,000 resampling iterations to estimate probability (Roff and Bentzen, 1989).

		A–D cluster						E–H cluster						
		Haplotype						Haplotype						
Collection	*n*	A	A′	B	C	C′	D	E	E′	F	G	H	Haplotype diversity	Nucleotide diversity
Hugh Smith River	10	3	0	0	4	1	0	2	0	0	0	0	0.78	1.31
Fish Creek (Taku River)	10	7	1	0	0	0	1	0	0	1	0	0	0.53	0.88
Ford Arm River	10	2	0	4	1	0	0	2	0	0	1	0	0.82	1.65
Crooked Creek	10	5	0	0	4	0	0	1	0	0	0	0	0.64	0.78
Eek River	10	0	0	0	4	0	0	4	0	0	0	2	0.71	1.87
Delta Clearwater River	10	0	0	0	0	0	0	0	0	0	0	10	0.00	0.00
Kamchatka River	10	5	0	0	4	0	0	0	1	0	0	0	0.06	0.94
Total	70	22	1	4	17	1	1	9	1	1	1	12	0.80	1.70
													(±0.024)	(±0.000)
Average													0.59	1.06
													(±0.11)	(±0.24)

collections from individual drainages ranged from 0.00 to 0.82 and nucleotide diversities ranged from 0.00 to 1.87 substitutions per 1000 bp. The distribution of haplotypes among collections was highly heterogeneous, which is noteworthy given the small sample sizes (n=10) (Table 3).

Phylogenetically, there were two clusters of haplotypes (haplotypes A–D and haplotypes E–H) (Fig. 3). Haplotypes of the two clusters differed by five or more restriction sites, and the average nucleotide divergence between individual haplotypes in the two clusters averaged 2.72 nucleotide substitutions per 1000 nucleotides, as compared to an average nucleotide diversity within each cluster of 0.87. The cluster of A–D accounted for the majority of fish, but haplotypes of both clusters were observed in collections from all drainages, except for fish from the Delta Clearwater River, which had only haplotype H. Bootstrap estimates of nucleotide divergence between clusters and average nucleotide diversity within clusters, estimated from the entire sample of 70 fish, were 2.98 ±0.06 and 0.35 ±0.05 (substitutions per 1000 nucleotides), respectively.

Expanded coho mtDNA survey based on variable sites

We increased the number of populations surveyed to 13 and increased sample sizes to 20, except for the Kam-

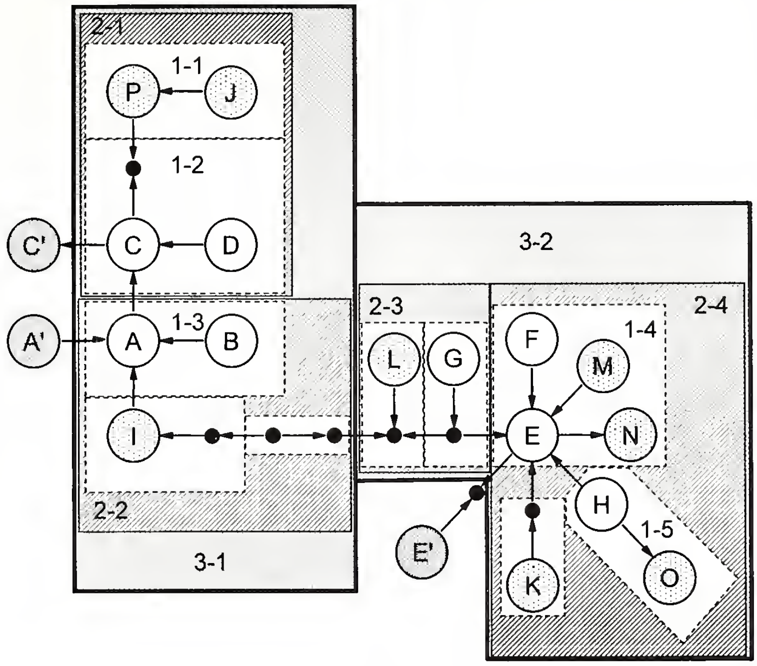

Figure 3

Haplotype tree for coho salmon mtDNA restriction site variation. Haplotypes A–H, including A′, C′, and E′, were observed in the preliminary survey (70 fish); haplotypes I–O were observed in the subsequent survey (188 additional fish); and haplotype P was detected in digests conducted to resolve positions on the tree of the latter haplotypes. Arrows indicate the direction of site gain. Ambiguities in the topology were resolved (Castelloe and Templeton, 1994) and the nested structure was constructed (Templeton and Sing, 1993). Haplotypes A′, C′, and E′ were pooled with A, C, and E, respectively, for the nested clade analysis. The results of the analysis are provided in Table 5.

chatka River (n=17) and Delta Clearwater River (n=21) populations. To make analysis of larger numbers of samples practical, we focused on restriction sites in five mtDNA regions that defined the most abundant eight (A–H) of the 11 haplotypes. Haplotypes A′, C′, and E′, each of which was represented by a single individual, were eliminated. In this survey, we analyzed site variation for the following PCR region by restriction endonuclease combinations:

12S/16S rRNA	Cyt b/D-loop	ND5/ND6	ND1/ND2	ND3/ND4
Cfo I	*Bst*N I	*Ava* I	*Dde* I	*Cfo* I
Dde I		*Dde* I	*Sty* I	

This survey detected 62 restriction sites (56.47 on average in each haplotype), which correspond to 262.67 nucleotides (238.22 on average). The proportion of the genome screened was a maximum of 1.58% (1.43% on average). To completely resolve the placement of haplotypes I, J, K, and L in the "gene tree," we digested their ND5/ND6 regions with *Mbo* I and their COI/COII regions with *Dde* I. An additional haplotype (P) was resolved. In total, the expanded survey resolved three additional haplotypes (I, J, and P) within the A–D clade and five additional haplotypes (K, L, M, N, and O) within the E–H clade (Table 4, Appendix 2, and Fig. 3). Haplotypes of both clusters appeared in most drainages. It is notable that four drainages included haplotypes of only a single clade: Delta Clearwater had 20 of haplotype H and one of the related haplotype O; Indian River had 19 of haplotype A and one of haplotype C; Berners River had five of hapotype A, 11 of haplotype C, one of haplotype I, two of haplotype J, and one of haplotype P; and the Little Susitna collection had four A haplotypes, 15 C haplotypes, and one I haplotype. Within drainages, haplotype diversities ranged from 0.10 to 0.73 and nucleotide diversities ranged from 0.22 to 9.07 substitutions per 1000 bp. The 13 collections exhibited strong heterogeneity ($P<10^{-4}$, Table 4).

In a Fitch-Margoliash phenogram that estimates relationships among drainages based on haplotype frequencies (Fig. 4), Delta Clearwater River differed strongly from the other collections, and the collections from systems that drain into the Bering Sea and from Karluk Lake on Kodiak Island, clustered separately. The remaining col-

Table 4

Observed numbers of each mtDNA haplotype, haplotype diversity, and nucleotide diversity (substitutions per 1000 bp) within collections of North Pacific coho salmon screened for variable sites detected in a preliminary survey (Table 3)(Nei and Tajima, 1983; Nei, 1987). Standard errors are in parentheses. Homogeneity of haplotype frequencies ($P_{MC} < 10^{-4}$) was tested by using Monte-Carlo simulation based on 10,000 resampling iterations to estimate probability (Roff and Bentzen, 1989).

		Haplotype																	
Collection	*n*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Haplotype diversity	Nucleotide diversity
Hugh Smith River	20	6	0	12	0	2	0	0	0	0	0	0	0	0	0	0	0	0.57	3.64
Fish Creek (Taku River)	20	11	0	3	2	0	2	0	0	0	1	0	1	0	0	0	0	0.68	5.68
Berners River	20	5	0	11	0	0	0	0	0	1	2	0	0	0	0	0	1	0.64	2.40
Indian River	20	19	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10	0.22
Ford Arm River	20	10	6	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0.68	4.51
Crooked Creek	20	9	0	10	0	1	0	0	0	0	0	0	0	0	0	0	0	0.57	2.50
Little Susitna River	20	4	0	15	0	0	0	0	0	1	0	0	0	0	0	0	0	0.42	1.13
Buskin River	20	5	0	12	0	3	0	0	0	0	0	0	0	0	0	0	0	0.58	4.69
Karluk River	20	1	0	9	0	5	0	0	0	0	0	1	0	0	4	0	0	0.73	9.07
Eek River	20	1	0	7	0	9	0	0	3	0	0	0	0	0	0	0	0	0.68	8.38
Kanektok River	20	5	0	8	0	5	0	0	1	0	0	0	0	1	0	0	0	0.75	7.91
Delta Clearwater River	21	0	0	0	0	0	0	0	20	0	0	0	0	0	0	1	0	0.1	0.2
Kamchatka River	17	9	0	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0.58	2.7
Total	258	85	6	96	2	28	2	1	24	2	3	1	1	1	4	1	1	0.734	6.73
																		(±0.016)	(±0.00)
Average																		0.54	4.08
																		(±0.06)	(±0.83)

lections spanned the southern portion of the geographic range from southern Southeast Alaska to the Kamchatka Peninsula. Within that set of collections, the two coastal Southeast Alaskan collections (Ford Arm and Indian River) appeared to form a weak cluster, but there was no obvious structure among the remaining collections.

We conducted AMOVA analyses reflecting a geographical hierarchy: Southeast Alaska, Southcentral Alaska, western and interior Alaska (Bering Sea), and Asia to examine the geographic basis of variation. Although the Karluk River collection resembled Bering Sea collections more than other northern Gulf of Alaska collections, we included it with the Southcentral Alaska group to maintain the geographic basis of the analysis. Analyzing the data based on haplotype frequencies (analogous to allelic differences in analysis of variance described by Weir [1996]) revealed highly significant divergence among collections (Φ_{ST}=0.291, P_{MC}<0.0001), most of which is attributable to average divergence among drainages within a region (Φ_{SC}=0.227, P_{MC}≤0.0001), rather than differences between regions (Φ_{CT}=0.083, P_{MC}=0.094). Incorporating relationships between the haplotypes into the analysis increased the proportion of the total divergence observed among drainages (Φ_{ST}= 0.449, P_{MC}≤0.0001) and among drainages within regions (Φ_{SC}=0.273 , P_{MC}<0.0001). The estimate of the proportion of divergence among regions also increased (Φ_{CT}=0.242, P_{MC}=0.083). Estimates of long-term gene flow $[N_{e(f)}m=(\Phi_{XY}^{-1}-1)/2]$ from Φ_{SC} and Φ_{CT} based on haplotype relationships were about 1 female per generation between collections within regions and about two females per generation between regions. Such estimates assume that an equilibrium between gene flow and random drift exists.

The nested clade analysis (Fig. 3, Table 5) collapses the "gene tree" from the periphery and analyzes each subclade for significantly small or large geographical distributions of the components as compared with the subclade as a whole. The first two levels of nesting are 1-step clades (nesting 0-step haplotypes) and 2-step clades (nesting 1-step clades). The significance in their geographic distributions were consistent with restricted gene flow with isolation by distance for haplotypes of the A–D (plus I, J, and P) subclade and past fragmentation for haplotypes of the E–H (less G plus M, N, and O) subclade. The 3-step clade (nested 2-step clades) that includes all of the E–H (plus L, M, N, and O) haplotypes also indicates past fragmentation. The most interior level of nesting, which contrasts the A group of haplotypes with the E group, assuming that the A group is interior (ancestral) based on the interpretations of Castelloe and Templeton (1994), is consistent with contiguous range expansion. We reanalyzed the data without the Delta Clearwater population, the Kamchatka population, and without either. These two geographically distinct populations did not alter the overall interpretation, but the Delta Clearwater population was responsible for significance of clade 2–4 and the Kamchatka population was responsible for the significance of clade 2–2.

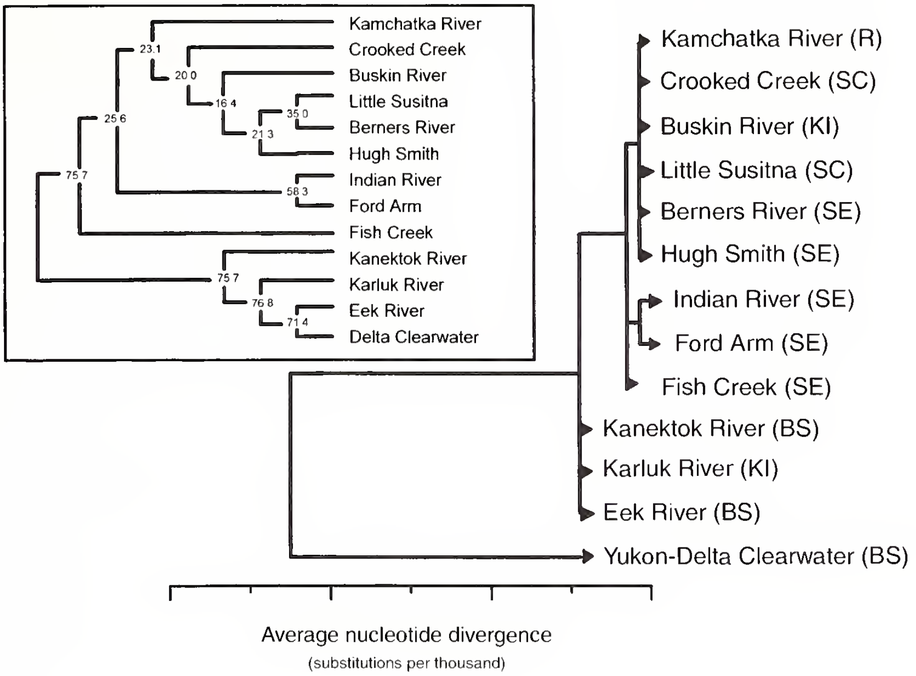
Kamchatka River
Crooked Creek
Buskin River
Little Susitna
Berners River
Hugh Smith
Indian River
Ford Arm
Fish Creek
Kanektok River
Karluk River
Eek River
Delta Clearwater
23.1
20.0
16.4
35.0
21.3
25.6
75.7
58.3
75.7
76.8
71.4
Kamchatka River (R)
Crooked Creek (SC)
Buskin River (KI)
Little Susitna (SC)
Berners River (SE)
Hugh Smith (SE)
Indian River (SE)
Ford Arm (SE)
Fish Creek (SE)
Kanektok River (BS)
Karluk River (KI)
Eek River (BS)
Yukon-Delta Clearwater (BS)
Average nucleotide divergence
(substitutions per thousand)

Figure 4

A Fitch-Margoliash least-squares phenogram constructed from nucleotide divergences among coho salmon collections and a consensus tree (inset) derived from 2000 iterations (Hedges, 1992) of bootstrap resampling from the individuals within each collection. Geographic regions for the samples are Russia (R), Southcentral Alaska (SC), Kodiak Island (KI), and Southeast Alaska (SE), and Bering Sea (BS), The consensus tree is unscaled and describes the confidence of the topology depicted in the phenogram.

Discussion

Coho salmon mtDNA variation

We examined the coho salmon mtDNA genome using restriction analysis of seven PCR products and detected fragments as small as 25 bp, which made it possible to sample nearly all (over 97%). of the mitochondrial genome. We are unaware of other fish species that have been as thoroughly surveyed. Our estimates of species-level parameters of molecular evolution should be quite robust in comparisons with those for other taxa, including fish.

Our results (Table 2) suggest that mtDNA variation is not evenly distributed throughout the genome and that focusing analysis on variable sites overestimates nucleotide diversity estimates (see Tables 3 and 4). Many previous studies of Pacific salmon species have limited the portion of the mtDNA genome surveyed (e.g. chum salmon [Cronin et al.; 1993; Park et al., 1993; Seeb and Crane, 1999]; sockeye salmon [Bickham et al., 1995; Taylor et al. 1996]; chinook [Cronin et al., 1993]). Other studies have included a limited geographic range of samples and have restricted the portion of the mtDNA genome studied (e.g. sockeye [Burger et al., 1997]; chinook [Adams et al., 1994]) or surveyed selected variable sites (e.g. pink salmon [Brykov et al., 1996; Seeb et al., 1999]; chum salmon [Scribner et al., 1998]; sockeye [Taylor et al., 1997]). Consequently, no previous studies have produced results that are appropriate for a broad comparison with our results.

We evaluated our results in the context of other fish species by plotting the effective number of haplotypes observed (the number of haplotypes which, if equally abundant, would result in the observed haplotype diversity, n_c, against the average nucleotide divergences between haplotypes for piscine species. For these comparisons, we used only data that were derived from 20 or more individuals and that surveyed the entire mtDNA genome (Fig. 5). Only two studies of Pacific salmon met those criteria; and our estimates of nucleotide diversity (0.4–4.5 per 1000 nucleotides) were generally lower than estimates for chinook salmon (6 haplotypes: 1.3–8.1; Wilson et al., 1987) and chum salmon (2 haplotypes: 2.4; Thomas et al., 1986).

Average nucleotide divergences between coho salmon haplotypes are quite low in relation to those of most other species studied; whereas, the effective number of haplotypes is near the median. The effective number of (selectively neutral) haplotypes (n_c) is monotonically related to the product of effective population number (of females

Table 5

Geographical nested clade analysis (Templeton, 1998) for Alaskan coho salmon mtDNA haplotpes based on the haplotype network in Figure 3 using GEODIS 2.0 (Posada et al., 2000). Only clades that exhibit significant results are shown. The analysis examines the geographical spread (D_C) of tip (T) and interior (I) subclades (Castelloe and Templeton, 1994), their relative spread within the clade (D_N), and contrasts their distrubutions (I–T) identifying distances that are significantly small (S), significantly large (L), or not significant (NS). Interpretations are based on Templeton's (1998) inference key.

1-step clades					2-step clades					3-step clades					4-step clades				
Clade	Sub-clades	Tip/Interior	D_C	D_N	Clade	Sub-clades	Tip/Interior	D_C	D_N	Clade	Sub-clades	Tip/Interior	D_C	D_N	Clade	Sub-clades	Tip/Interior	D_C	D_N
1-3	A	I	NS	NS	2-1	1-2	I	NS	NS	3-2	2-3	I	NS	L	4-1	3-1	I	S	S
	B	T	S	S		1-1	T	S	NS		2-4	T	S	S		3-2	T	NS	L
		I–T contrast	L	L			I–T contrast	L	NS			I–T contrast	NS	L			I–T contrast	NS	S
	Isolation by distance.					Isolation by distance.					Past fragmentation					Contiguous range expansion			
1-4	E	I	NS	NS	2-2	1-3	I	L	L										
	F	T	NS	L		I	T	NS	NS										
	M	T	NS	NS			I–T contrast	NS	NS										
	N	T	S	S		Islation by distance.													
		I–T contrast	L	NS															
	Past fragmentation				2-4	1-4	I	NS	L										
						1–5	T	S	NS										
						K	T	NS	NS										
							I–T contrast	L	NS										
						Past fragmentation													

in the case of mtDNA – $N_{e(f)}$) and mutation rate (μ) (Kimura and Crow, 1964; Ohta and Kimura, 1973). However, for μ that are relatively constant among species, n_c orders the relative size of historic effective sizes of the species (Avise et al., 1988). Estimates of n_c may be influenced by the number of individuals analyzed, the scope of sampling with respect to both geographic range, and the number of sites detected in the mtDNA genome.

The average nucleotide sequence divergence between haplotype pairs presents a more complex picture. Accumulation of nucleotide differences between haplotypes requires time. If the mutation rates (μ) are reasonably similar (although there is debate about the degree of similarity [e.g. Avise et al., 1988; Gibbons, 1997]), the extent of haplotype divergence should order the species according to the time that has elapsed since a major species bottleneck. Such ordering is a consequence of lineage sorting within isolates, which tends toward a set of related (monophyletic) haplotypes but fosters divergence among isolates. However, as a result of lineage sorting, species composed of isolates are likely to exhibit higher average divergence than species broadly connected by gene flow (Neigel and Avise, 1986). The small nucleotide divergence among haplotypes in coho salmon suggests that extant coho salmon share a relatively recent common female ancestor.

Two distinct clusters of haplotypes account for most of the divergence observed among coho salmon. The clusters are separated by an average of 2.72 substitutions per 1000 nucleotides, but divergence among haplotypes within each cluster average about 0.87 substitutions per thousand nucleotides.

Expanded coho mtDNA survey from variable sites

By concentrating our effort on variable sites, we evaluated the variation present in a larger number of fish and from additional populations. The two clusters of haplotypes observed in the preliminary survey persisted, each augmented by several new haplotypes. The average divergence between haplotypes within each cluster was 4.95 substitutions per 1000 nucleotides and the clusters averaged 10.28 substitutions per 1000 bp apart. By focussing on variable restriction sites, estimates of nucleotide divergence were inflated more than threefold. However, the data remain useful for examining intraspecific structure, and strong heterogeneity among collections indicated structure among populations or at higher levels of population hierarchy.

The unrooted tree (Fig. 4) depicting relationships among collections shows that the Bering Sea and the Karluk River collections cluster separately from the other collections. The cluster-based structure of the "gene tree" accentuates the divergence between geographic regions because the highest abundance of E-cluster haplotypes occurs in the Bering Sea (and Karluk) collections. In fact, the Delta Clearwater collection is fixed for E-cluster haplotypes. The AMOVA analyses revealed a population structure that appears stronger within regions than among regions. Both estimates of gene flow (one to two females per generation) are low in relation to allozyme-based estimates of other Pacific salmon species (i.e. an exchange of 6.6 to 16.4 individuals per generation for pink salmon [McGregor, 1983; Beacham et al., 1985; Noll et al., 2001]; 6.3 to 9.0 for chum salmon [Kondzela et al., 1994; Phelps et al., 1994; Wilmot et al., 1994; Winans et al.. 1994]; 1.3 to 7.1 for sockeye salmon [Wood et al., 1994; Varnavskaya et al., 1994]; and 1.2 to 4.0 for chinook salmon [Gharrett et al., 1987; Utter et al., 1989; Bartley and Gall, 1990]) but consistent with estimates from coho salmon allozyme data (Wehrhan and Powell, 1987; Bartley et al., 1992).

It is essential to keep in mind that such gene-flow estimates assume an equilibrium between gene flow and random drift. The distribution of coho salmon haplotypes suggests that a broad equilibrium may not exist. For example, the Delta Clearwater River population was nearly fixed for haplotype H, an E-cluster haplotype found only in the Bering Sea populations. Its haplotype composition reflects its geographic isolation from other coho populations. Also, the haplotype distribution from the Kamchatka River population was surprising because it is so similar to Southeast Alaskan populations, but differed from the geographically closer Bering Sea populations. This makes sense if the genetic structure of extant coho salmon populations is strongly influenced by historic events, such as historic random drift, colonization from eastern populations, or survival in an different glacial refugia, and that an equilibrium between gene flow and random drift has not yet been reached.

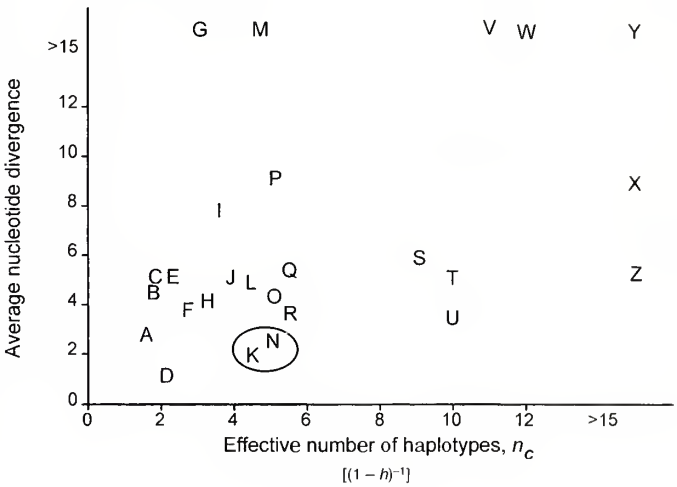

Figure 5

Comparison of the effective number of haplotypes (n_c) and the average nucleotide divergence between haplotypes within a species for 25 species of fish estimated from haplotype diversity (h). The number of effective haplotypes is monotonically related to $N_e\mu$ (μ is the mutation rate); larger average nucleotide divergences require longer times and reduced gene flow to develop. The following species were plotted: A = red grouper (Richardson and Gold, 1993, 1997); B = white marlin (Graves and McDowell, 1995); C = hardhead catfish (Avise et al., 1987, 1989); D = American eel (Avise et al., 1986, 1989); E = oyster toadfish (Avise et al., 1987, 1989); F = American shad (Bentzen et al., 1989); G = redear sunfish (Avise et al., 1987, 1988, 1989, 1992); H = *Sicyopterus stimpsoni* (Zink et al., 1996); I = lake whitefish (Bernatchez and Dodson, 1991); J = red snapper (Gold et al., 1994, 1997); K = coho salmon (Carney et al., 1997); L = black drum (Richardson and Gold, 1993; Gold et al., 1994); M = spotted sunfish (Avise et al., 1987, 1989, 1992; Avise, 1992); N = coho salmon (this study); O = NW Atlantic capelin (Dodson et al., 1991); P = bowfin (Avise et al., 1987, 1989, 1992; Avise, 1992); Q = striped marlin (Graves and McDowell, 1994; Graves and McDowell, 1995); R = *Stenogobius hawaiiensis* (Zink et al., 1996); S = *Lentipes concolor* (Zink et al., 1996); T = greater amberjack (Richardson and Gold, 1993); U = *Awaous guamensis* (Zink et al., 1996); V = Atlantic herring (Kornfield and Bogdanowicz, 1987; Richardson and Gold, 1993); W = largemouth bass (Nedbal and Phillipp, 1994); X = red drum (Gold and Richardson, 1991; Gold et al., 1993); Y = warmouth (Avise et al., 1987, 1989, 1992; Avise, 1992); Z = NE Atlantic capelin (Dodson et al., 1991). Estimates for coho salmon in this study (N) and our previous study (K) are circled.

Synthesis

The data obtained from restriction site analysis provide a present day "snapshot" of coho salmon mtDNA variation and have two levels of resolution. One level is the geographic distribution of haplotypes and the haplotype compositions of the populations sampled. The other level is the pattern and extent of divergence among the haplotypes. The mtDNA haplotype compositions of populations indicate that coho salmon, at least in their Alaskan range, generally have lower gene flow than other Pacific salmon species, although the comparisons assume equilibria between gene flow and random drift that may not yet have been reached. Coho salmon exhibit divergence among populations within regions, but generally not fixed differences. Many of the haplotypes were found in populations throughout the range examined, which suggests that at some time the haplotypes were disseminated either through gene flow or colonization. Our data, are generally consistent with the known distributions and abundances of North Pacific coho salmon, that is, coho salmon is a species composed of many small spawning populations that have low levels of gene flow. The low geneflow rates estimated within several geographic ranges indicate that a finer scale study of coho salmon population structure is warranted.

Superimposed on this "snapshot" of population structure is the mtDNA "gene tree," which carries information about the demographic history of coho salmon. Nested clade analysis of the geographic distribution of haplotypes and clades of related haplotypes reveal recent isolation by distance, particularly for the geographical distribution of A-cluster haplotypes. There is also evidence of past population fragmentation in the distribution of E-cluster haplotypes, and the interior of the tree has evidence of range expansion. The topology of the tree also indicates recent demographic expansion preceded by stationary, or more likely, declining populations. Radiation of haplotypes to form a star-like pattern occurs when populations expand (Slatkin and Hudson, 1991), such as the haplotypes that surround haplotypes A and E. Whereas, in stationary or declining populations, the haplotype composition of a population eventually tends toward a single, evolutionarily related (monophyletic) set as a result of stochastic processes (Neigel and Avise, 1986), such as the A- or E-cluster.

It is likely that the demographic history of coho salmon is closely tied to Pleistocene events. During the Pleistocene Epoch, glaciers periodically advanced and retreated in regions bordering the northeastern Pacific Ocean. These advances were accompanied by a drop in sea level (exceeding 100 m), decreased sea surface temperatures, increased coverage of sea ice, and reduced marine surface water productivity (e.g. Porter, 1989; Bartlein et al., 1991; de Vernal and Pedersen, 1997). Much of the present day freshwater range was physically covered with ice (Hamilton, 1986). During the last glacial maximum, coho salmon must have been displaced from most of British Columbia and the Gulf of Alaska coast, now the center of their range. During each glacial advance, it is likely that marine and freshwater habitats of Pacific salmon populations were greatly reduced, especially for species that require freshwater rearing, such as coho salmon. Alteration and reduction of natural ranges during these advances probably resulted in the declines or extirpation of many populations. The patterns observed in the analysis of coho salmon mtDNA variation resulted from major demographic changes involving the range of drainages sampled and are consistent with the effects that would be expected as a result of glacial advances and retreats. For most of the last 500,000 years, the environment was much harsher than now, as indicated by the sea level (a measure of the amount of the world's water tied up in ice), which was more than 50 m lower than today during most of that period (Porter, 1989; Bartlein et al., 1991). Peaks of recent interglacial periods during which the environment probably approached modern conditions have occurred about every 100 thousand years, approximately 120 thousand years ago (120 ka) , 200 ka, 330 ka, and 400 ka (Porter, 1989; Petit et al., 1999).

A linkage between geologic record and molecular evolution requires application of a "molecular clock" to relate observed nucleotide divergences to a mutation rate. The calibration of mtDNA clocks is contentious. A rate of 2% nucleotide substitution per million years (Brown et al., 1982), referred to as the "standard clock," has been broadly applied. However, the rates for salmonids may be slower, and rates of 1% per million years or less (Smith, 1992) may be more appropriate.

An interpretation of our results based on the "standard clock" would be that divergence between clusters began 136 ka at the beginning of the last interglacial period and that divergence within the clusters began 43 ka, at the end of the last interglacial period. The small number of haplotypes within each cluster is consistent with a relatively small effective number of females ($N_{e(f)}$) within refugia followed by rapid expansion or the existence of several isolates within each refugium. Following the last glacial maximum, dispersion from one or more glacial refugia was followed by incomplete inter-refugial introgression. The objection to interpretations based on the "standard clock" is that the divergence rate is much faster than the rate generally accepted for salmonids. However, mtDNA clocks are calibrated from interspecies comparisons, which occur over times measured in millions of years (Thomas and Beckenbach, 1989; Martin et al., 1992; Bentzen et al., 1993; Phillips and Oakley, 1997). The mutation rate is certainly not homogeneous over the mtDNA genome, so it is possible that mutational hot spots dominate the rate in shorter time spans but are saturated and much less important in estimates over long time spans.

Although there are unquestionably strong demographic signals in the molecular evolutionary record of coho salmon, we can not match them unequivocally to specific Pleistocene events. To make such a match, several pieces of information are needed. First, the data available for calibrating a clock for the entire salmonid mtDNA genome are limited. Although there are numerous studies of particular mtDNA regions, rate differences among regions compromise their utility. Second, short-term, intraspecific clocks based on hot spots must be developed and compared with long-term, interspecific clocks. We are now examining data from other Pacific salmon species that share the northern Pacific Ocean range with coho salmon. Concordance among those results interpreted from the well-described Pleistocene environmental history may provide us with independent records of Pleistocene influences on the demography of Pacific salmon, which can be used to calibrate intraspecific mtDNA clocks.

The haplotype compositions of the populations that we studied leave us with questions about patterns of post-glacial colonization and influences of the Cascadian and Beringian refugial stocks. Acquisition and analysis of samples from native populations in the Pacific Northwest should resolve questions about the composition of coho salmon in the Wisconsin Cascadian refugium. And, an intensive study of populations skirting the Alaska Peninsula and eastern Bering Sea should provide information about local colonization patterns and the composition of Beringian coho.

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Appendix 1

Restriction fragment sizes for seven PCR-amplified coho salmon mtDNA regions for twelve restriction endonucleases. Composite haplotypes are shown in Appendix 2.

2S/165

Ase I	*Ava* I	*Bst*N I	*Bst*U1	*Cfo* I		*Dde* I			*Hind* II	*Hinf* I	*Mbo* I		*Msp* I	*Rsa* I	*Sty* I
1	1	1	1	1	2	1	2	3	1	1	1	2	1	1	1
2400	1200	1000	1300	1210	1100	455	400	400	750	1800	1475	900	950	930	1500
	1200	410	475	380	380	400	280	280	720	600	375	575	650	600	500
		380	300	350	350	280	250	205	720		210	375	400	290	200
		310	275	150	150	155	205	205	210		170	210	190	200	200
		200		120	120	145	155	155			170	170	130	200	
		150			110	130	145	145				170	80	180	
						125	130	130							
						120	125	125							
						104	120	120							
						80	104	104							
						65	80	80							
						55	65	65							
						48	55	55							
						45	48	48							
						37	45	45							
						35	37	45							
						33	35	37							
						30	33	35							
						27	30	33							
							27	30							
								27							

ND1/ND2

Ase I	*Ava*I	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I			*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I			
1	1	1	1	1	1	2	3	1	1	1	1	1	1	2	3	4
1786	1596	1496	2026	1146	780	780	780	1846	1546	1026	1926	1176	650	650	550	550
520	600	1150	500	900	480	480	480	800	850	590	780	375	550	550	530	530
230	450		120	600	240	240	240		250	510		275	530	530	470	470
110					220	210	180			310		250	395	305	395	305
					180	180	148			210		220	305	305	305	305
					145	145	145					220	230	230	230	230
					123	123	123					130		90	180	180
					120	120	120									90
					115	115	115									
					95	95	95									
					78	78	78									
						(10)	62									
							(10)									

continued

Appendix 1 (continued)

A8/A6/COIII/ND3

Ase I	*Ava* I	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
1	1	1	1	1	1	1	1	1	1	1	1
2210	2210	1200	1700	1300	970	1300	500	1800	800	780	500
		700	500	900	970	900	498	210	600	680	450
		310			180		400	200	490	345	350
					80		300		320	240	340
							160			150	240
							150				220
							150				90

COI/COII

Ase I	*Ava* I	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I			*Hind* II	*Hinf* I		*Mbo* I		*Msp* I	*Rsa* I	*Sty* I
1	1	1	1	1	1	2	3	1	1	2	1	2	1	1	1
1562	2432	1152	1422	1522	530	530	530	1682	575	500	650	550	2000	872	1282
650		890	500	1010	360	360	360	750	500	500	550	520	220	350	1000
220		390	410		330	330	330		410	410	480	480	212	350	150
					293	293	293		295	295	270	270		300	
					255	255	255		150	150	220	220		225	
					235	235	235		150	150	175	175		215	
					196	196	209		150	150	75	130		120	
					119	110	110		90	90		75			
					110	80	80			75					
					26	39	39								
					(13)	26	26								
						(13)									

ND3/ND4

Ase I	*Ava* I	*Bst*N I	*Bst*U I	*Cfo* I		*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
1	1	1	1	1	2	1	1	1	1	1	1	1
1955	2105	1050	1800	1600	1100	525	2305	1730	525	900	875	1970
350	200	750	500	700	700	490		550	500	800	490	330
		500			500	400			400	300	365	
						290			350	250	230	
						150			190	115	140	
						150			190		110	
						90			150		90	
						90						

ND5/ND6

Ase I	*Ava* I		*Bst*N I		*Bst*U I	*Cfo* I	*Dde* I				*Hind* II	*Hinf* I	*Mbo* I		*Msp* I	*Rsa* I	*Sty* I
1	1	2	1	2	1	1	1	2	3	4	1	1	1	2	1	1	1
1100	2530	2230	900	900	2025	1000	665	600	600	600	1950	550	1442	1442	1525	2530	1675
850		300	900	900	525	800	490	570	490	490	550	475	410	410	410		600
550			650	400		500	300	490	320	300		420	312	312	285		275
			100	250		200	270	320	300	270		320	286	281	200		
				100			200	160	270	200		275	100	100	130		
							160	150	160	160		210		(5)			
							150	145	150	150		175					
							145	65	145	145		125					
							120		65	120							
										65							

continued

Appendix 1 (continued)

CytB/D-Loop													
Ase I	*Ava* I	*Bst*N I			*Bst*U I	*Cfo* I	*Dde* I	*Hind* II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	*Sty* I
1	1	1	2	3	1	1	1	1	1	1	1	1	1
1524	1534	900	900	900	2100	900	550	2094	690	810	700	1240	1050
600	520	850	540	540	444	694	430	450	650	610	330	400	580
420	315	415	415	400		510	310		470	400	240	390	450
	175	400	400	310		300	290		310	170	225	195	220
		130	310	220		140	280		250	165	210	140	140
			130	195			280		230	165	205	125	110
				130			175			150	205	105	70
							130			72	180		
							90				180		
											120		

Appendix 2

Key to haplotype composition inferred from variable fragment patterns in Appendix 1. No variation was observed in the A8/COIII region (Table 2). Numbers in parentheses are presumed haplotypes.

	ND1/ND2		12S/16S			ND3/ ND4	Cytb/ Dloop	ND5/ND6				COI/A8		
Haplotype	*Dde* I	*Sty* I	*Cfo* I	*Mbo* I	*Dde* I	*Cfo* I	*Bst*N I	*Ava* I	*Bst*N I	*Dde* I	*Mbo* I	*Dde* I	*Hin*F I	*Mbo* I
A	2	4	2	1	2	1	2	2	2	2	2	2	2	1
A′	2	4	2	1	2	1	2	2	1	2	2	2	2	1
B	2	4	1	1	2	1	2	2	2	2	2	2	2	1
C	2	4	2	1	2	1	3	2	2	2	2	2	2	1
C′	2	4	2	2	2	1	3	2	2	2	2	2	2	1
D	2	3	2	1	2	1	3	2	2	2	2	2	2	1
E	3	2	2	1	2	1	1	1	2	4	1	2	2	1
E′	3	2	2	1	2	1	1	1	2	4	1	2	1	2
F	3	1	2	1	2	1	1	1	2	4	1	2	2	1
G	3	2	2	1	2	1	1	1	2	3	1	1	2	1
H	3	2	2	1	1	1	1	1	2	4	1	2	2	1
I	2	4	2		2	1	1	2		2	2	2		
J	2	4	2		2	1	3	1		3	2	3		
K	1	2	2		2	1	1	1		4	2	2		
L	3	1	2		2	1	1	1		3	1	2		
M	3	2	2		2	1	1	1		1	(1)	(2)		
N	3	2	2		3	1	1	1		5	(1)	(2)		
O	3	2	2		1	2	1	1		5	(1)	(2)		
P	2	4	2		2	1	3	1		3	2	2		

Diet and consumption rates of overwintering YOY striped bass, *Morone saxatilis*, in the Hudson River*

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Abstract–Starvation is often proposed as the mechanism of size-dependent mortality of overwintering temperate fishes, yet little is known about the energetics of fish at low temperatures. Young-of-the-year (YOY) Hudson River striped bass (*Morone saxatilis*) suffer a winter energy deficit and experience size-selective winter mortality in some years that may influence recruitment. To better understand the role of energetic stress in winter mortality, we determined diet composition and measured consumption rates of wild fish. Gastric evacuation rates were measured in the laboratory at 2°, 5°, 8°, and 11°C. Measured evacuation rates for YOY striped bass were among the lowest reported, with 25% of the initial meal remaining after 150 hours at 2°C. Variation in evacuation rate data increased as temperature decreased. Diets of fish captured in winter were dominated by gammarid amphipods and shrimp species. Gut fullness ranged from 0% to 7%, averaging 0.4% body weight. Evacuation rates, gut fullness values, and river temperatures were combined to estimate daily consumption rates on 29 dates over five winters. Consumption estimates ranged from 0% to 0.29%bw/day and were generally higher in early winter than in late winter and not correlated with river temperatures. Stomach fullness was negatively correlated with the level of lipid energy reserves at the individual level. The patterns may reflect an internal control on appetite, or a depression of prey availability in late winter. These findings indicate that the potential for winter starvation may be influenced by both internal and external constraints on consumption rate.

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Although the feeding and growth of many temperate fish species have been extensively examined during summer months, details of the feeding ecology of most species during winter remain largely unknown. Knowledge of the winter energetics of many fish species is restricted to the observation that growth decreases markedly in autumn and often becomes negligible during winter. There are only scattered reports of the feeding patterns of overwintering temperate fishes (Daiber, 1956; Keast, 1968; Foltz and Norden, 1977; Diana, 1979), and few estimates of consumption rates (but see Diana, 1979; Minton and McLean, 1982). This lack of information on the winter energetics of fishes applies also to laboratory-based physiological measurements, including gastric evacuation rates and metabolic rates. Despite the observation that temperature is the predominant factor regulating digestion, for none of the 22 species listed in a compilation of gastric evacuation data (He and Wurtsbaugh, 1993) had rates been measured at the minimum temperatures likely to be encountered in winter.

Many temperate fish species lose energy throughout the winter, relying upon stored lipid reserves to fuel metabolism (Toneys and Coble, 1980; Schultz and Conover, 1997; Hurst et al., 2000). Reliance on lipid reserves is believed to be necessary because consumption rate is severely limited by the ability of fish to digest food at low temperatures. However low consumption rates of overwintering fish may also be limited by low food availability (Cunjak and Power, 1987; Foy and Paul, 1999).

Winter mortality that selects against smaller fish has been documented in a number of temperate species (Sogard, 1997) and is most commonly attributed to depletion of lipid energy reserves and starvation. Smaller fish have higher starvation mortality rates because they tend to have lower energy reserves and higher weight-specific metabolic rates than larger fish. An implicit assumption in the "starvation hypothesis" is that overwintering fish have little or no capacity to obtain energy by feeding. However, in several experiments, fish allowed to feed *ad libitum* had higher winter survival rates than unfed fish (Post and Evans, 1989; Thompson et al., 1991; Hurst and Conover, 1998). Although wild fish are unlikely to have access to unlimited food, these results suggest that fish benefit from feeding during winter. Furthermore, some overwintering fish increase consumption rates in response to depletion of internal reserves (Metcalfe and Thorpe, 1992; Bull et al., 1996; Hurst and Conover, 2001). These findings suggest the need for a more complete understanding of the energetics of overwintering fish, including consumption rates of wild fish and an evaluation of factors that regulate consumption in winter.

The striped bass (*Morone saxatilis*) is a relatively well studied, commercially important species along the east coast of the United States and Canada (Boreman and Austin, 1985). Previous work has shown that recruitment to the Hudson River population may be regulated by the severity of the winter that age-0 fish encounter (Hurst and Conover, 1998). In addition, size-selective winter mortality has been documented among young-of-the-year (YOY) striped bass in both the Hudson River and Miramichi River populations (Hurst and Conover, 1998; Bradford and Chaput[1]).

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Young-of-the-year striped bass in the Hudson River experience a winter energy deficit that varies in severity among years (Hurst et al., 2000). This interannual variability may be related to feeding conditions in the environment. Although studies of the summer feeding habits of YOY striped bass are available from most east coast estuaries (Markle and Grant, 1970; Boynton et al., 1981; Gardinier and Hoff, 1982; Rulifson and McKenna, 1987), information for overwintering fish is extremely limited (Hartman and Brandt, 1995a). A bioenergetics model has been developed for juvenile striped bass (Hartman and Brandt, 1995b), describing metabolic rates and maximum consumption rates of fish across a wide range of temperatures. This type of deterministic bioenergetic modeling does not take into account the observed variability in energy storage and depletion cycles (Hurst et al., 2000) or compensatory feeding responses observed in response to depletion of reserves (Metcalfe and Thorpe, 1992). A better understanding of the energetics of overwintering juvenile striped bass, including *in situ* estimates of consumption rates, is required to fully evaluate the potential for winter starvation in this species.

In our study, we present results of experiments measuring gastric evacuation rates at winter temperatures necessary to determine consumption rates of wild fish. We also describe the diet of overwintering YOY striped bass and estimate consumption rates of overwintering fish on 29 dates over five winters. Finally, we analyze feeding patterns at both the individual and population level to determine the factors regulating consumption rates of overwintering fish and discuss these results as they relate to the potential for winter starvation.

Methods

Gastric evacuation experiments

Wild fish were captured from the Hudson River estuary and transported in river water to the Flax Pond Marine Laboratory of the State University of New York at Stony Brook in Old Field, New York. Fish were treated with 0.60 ppm copper sulphate for 5 minutes and 15 ppm oxytetracycline for 5 days to reduce risk of mortality from infection. Fish were acclimated to laboratory conditions for at least 3 weeks prior to use and only fish appearing healthy and behaving normally were used in the experiment. Temperatures during the acclimation period were maintained between 1° and 5°C, and salinities were maintained at 15 ppt. Fish were fed frozen adult brine shrimp (*Artemia* sp.) and sand shrimp (*Crangon septemspinosa*) daily during the acclimation period. Prior to experimentation, groups of fish were acclimated to the test temperature (±0.5°C) for at least one week. Fish rarely fed voluntarily at low temperatures and were not offered food for between 3 and 5 days prior to use in the experiment.

Evacuation rates were measured at 2°, 5°, 8° and 11°C. Temperatures in the experimental tanks were maintained by recirculating fluid chillers; they never differed from the prescribed temperature by more than 0.5° and were generally within 0.2°C. Fish used in the experiment ranged in size from 88 to 150 mm TL (5.6 to 31.4 g wet weight), the natural size range of YOY striped bass in winter. To reduce stress during the feeding process, fish were weighed (to 0.01 g wet weight) and acclimated to 65-L test tanks for 12 hours prior to feeding. Individual fish were captured with a dip-net from the test tank, force-fed the meal, and returned to the test tank within 1 minute. Fish were fed by opening the mouth and forcing the meal through the esophagus with a pair of blunt forceps. A meal comprised a single whole or partial *C. septemspinosa* weighing approximately 2% of the fish's body weight. A ration level of 2% body weight was chosen because it falls in the upper range of, but is well below the maximum, gut fullness levels observed among wild fish. Each fish remained in the test tank until the time of sampling, when it was netted and sacrificed with an overdose of MS-222 anesthetic and measured (to 1.0 mm TL). The remaining stomach contents were dissected from the fish, dried of excess water, weighed (to 0.001 g), and dried to a constant weight at 60°C. In no cases did fish regurgitate the meal following feeding or during the netting and sacrifice procedure.

The evacuation rate of at least 3 fish was measured at each of 10 or more time points at each temperature (min. 33 fish at 11°C; max. 43 fish at 2°C). The maximum interval between feeding and sacrifice encompassed the majority of the evacuation time at each temperature and ranged from 72 hours at 11°C to 168 hours at 2°C. The minimum interval used was 0.25 hours at all temperatures. Fish were exposed to a 10:14 light:dark cycle to mimic the natural winter photoperiod. Feeding time was standardized to the light:dark cycle, and fish were exposed to light for the first 8–10 hours of digestion.

Evacuation of the meal was described with an exponential model allowing a time-lag prior to the beginning of evacuation because of its utility in estimating rations among wild fish (Elliott and Persson, 1978; Bromley, 1994). The model was fitted by using biphasic nonlinear regression of untransformed data on the percentage of initial meal remaining over time with the equation

$$\%\ remaining = \begin{cases} 100 & if\ \ t < (c_0 + c_1 T) \\ 100e^{-(b_0 e^{b_1 T})[t-(C_0+C_1 T)]} & if\ \ t \geq (c_0 + c_1 T), \end{cases}$$

where T = temperature;

t = time in hours since ingestion;

b_0 and b_1 = the coefficients of the exponential relationship between evacuation rate and temperature; and

---

[1] Bradford, R. G.,and G. Chaput. 1997. Status of striped bass (*Morone saxatilis*) in the Gulf of St. Lawrence in 1996 and revised estimates of spawner abundance for 1994 and 1995. Dep. Fish. and Oceans, Can. Stock Assess Secretariat Res. Doc. 97/16, 31 p. Dep. Fisheries and Oceans, Science Branch, Gulf Region, P.O. Box 5030, Moncton, New Brunswick E1C 9B6, Canada.

Table 1

Summary of collections and diets of overwintering YOY striped bass collected in the Hudson River Estuary, 1993–97. %F = mean frequency of occurrence, %W = mean percentage of wet weight; *n* = number of fish sampled per date.

		Winter									
		1993		1994		1995		1996		1997	
No. of dates sampled		4		8		7		6		4	
Temperature (°C)		2.0–5.1		4.2–10.4		1.0–9.4		3.3–6.2		5.0–6.1	
n		20–34		15–53		8–37		16–29		18–52	
Percentage empty stomachs		71.0		52.5		72.3		57.6		73.4	
Mean gut fullness ±SE		0.44 ±0.103		0.51 ±0.056		0.34 ±0.066		0.18 ±0.037		0.26 ±0.060	
Prey type	Scientific name	%F	%W	%F	%W	%F	%W	%F	%W	%F	%W
Amphipods	*Gammarus* sp.	66.7	59.1	92.1	90.2	58.2	39.5	86.3	52.4	34.3	15.7
Sand shrimp	*Crangon septimspinosa*	37.0	27.1	4.2	4.2	7.1	8.1	0	0	3.1	5.3
Grass shrimp	*Palaemonetes pugio,* *Palaemonetes vulgaris*	38.9	6.4	0	0	4.3	4.1	0	0	3.1	7.5
Mysid shrimp		0	0	0	0	0	0	3.0	7.1	18.8	4.3
Unidentifiable shrimp	*C. septimspinosa,* *P. pugio, P. vulgaris*	0	0	0.7	0.4	27.5	22.8	16.4	32.5	21.4	26.4
Crabs		33.3	5.7	0	0	0	0	0	0	0	0
Polychaetes		0	0	0	0	17.9	23.3	0	0	0	0
Oligochaetes		0	0	0	0	0	0	0	0	23.9	38.8
Fish	*Anchoa mitchilli,* *Menidia menidia,* *Ammodytes americanus*	1.9	1.7	5.4	5.3	2.9	2.2	7.1	8.0	3.1	1.9
UIR[1]		40.7	24.4	11.9	8.5	28.2	6.0	31.8	29.2	42.2	25.1

[1] UIR = unidentified invertebrate remains. These were not included in the calculation of %W of other prey items.

c_0 and c_1 = the intercept and slope of the linear relationship between temperature and the lag prior to the beginning of evacuation.

If no lag is present in the data, the parameters c_0 and c_1 approach 0. The effect of body size on evacuation was assessed by examining the relationship between fish length and deviation from the best fitting evacuation model.

Diet of YOY striped bass

Overwintering YOY striped bass were collected with a 9-m bottom trawl (38-mm stretch mesh codend) from the lower Hudson River estuary, the only known wintering aggregation for the Hudson River population (Dovel, 1992). Sampling occurred throughout the overwintering period of five consecutive years. Fish were captured during daylight hours between river mile 0 and 9 in conjunction with the New York Power Authority's hatchery evaluation and tagging program. Sampling in each year began in mid-December and ended in late March or early April. Bottom water temperatures were measured during sampling. The number of fish analyzed for diet per date ranged from 8 to 53 depending on catch rates (Table 1). Captured fish were individually wrapped and immediately frozen for preservation. In the laboratory, fish were thawed, measured (mm TL), weighed (g wet weight), and stomach contents were removed. Prey were identified to the lowest possible taxon and weighed. All prey in each category in each stomach were weighed as a group because the abundance and small size of the dominant prey item made individual measurements unfeasible (gammarid amphipods; often >50/stomach and <0.005 g each). Diets of YOY striped bass were described by the contribution of items expressed on the basis of both weight ($\%W_i = \textit{weight of prey}_i / \textit{weight of all identifiable prey}$) and frequency ($\%F_i = 100 \times \textit{no. of stomachs with prey}_i / \textit{no. of stomachs with prey}$). Unidentifiable items were measured separately and are expressed as a percentage of the total weight of all stomach contents.

Estimation of consumption rates

Daily consumption rate estimates were generated by combining gut fullness values (S=total weight of stomach contents/fish weight) of overwintering YOY striped bass with laboratory-determined gastric evacuation rates with the method of Eggers (1979):

$$C = 24R_e \times \overline{S},$$

where $\overline{S}$ = average gut fullness of field-caught fish; and
R_e = exponential evacuation rate at measured field temperatures determined from laboratory experiments described above.

This simplified version of the Elliott and Persson (1978) model is appropriate when sampling is not conducted in discrete time intervals. We used gut-fullness measures from fish collected throughout the day, as described above. Although fish were sampled only during daylight hours, the sampling interval (24 hours) is substantially shorter than the evacuation time (>72 hours), even at the warmest temperatures observed; hence any diel feeding patterns will have little effect on our consumption estimates.

Standard deviations of consumption estimates were generated from a Taylor expansion of the consumption model

$$\sigma_{\overline{C}}^2 = \left(\frac{d\overline{C}}{d\overline{S}}\right)^2 \sigma_{R_e}^2 + \left(\frac{d\overline{C}}{dR_e}\right)^2 \sigma_{\overline{S}}^2 + 2\left(\frac{d\overline{C}}{dR_e}\right)\left(\frac{d\overline{C}}{d\overline{S}}\right)\operatorname{cov}(R_e,\overline{S})$$

The variance in the evacuation rate parameter (R_e) was estimated through a bootstrap procedure by fitting the evacuation model to 1000 sets of 152 observations sampled with replacement from the evacuation rate data. Since R_e is a function of temperature, the variance in R_e at a given temperature was determined by inserting the temperature into the 1000 model fits. The variance in $\overline{S}$ was estimated for each date as var(S_i)/n.

The standard deviation of the consumption estimate depends upon the covariance between evacuation rate (R_e), measured in laboratory experiments, and gut fullness ($\overline{S}$), observed in wild fish. Because we have no way of measuring the covariance between these parameters, we evaluated the importance of this term by calculating the variance under three assumptions: 1) $\overline{S}$ and R_e are not correlated, 2) they covary perfectly, and 3) they display perfect negative covariance.

Analysis of feeding patterns

We investigated feeding patterns of YOY striped bass by examining the relationship between several factors (body size, time of year, water temperature, and energy storage) and gut fullness, at the individual and population level. Gut fullness was chosen over consumption rate for three reasons. First, gut fullnesses were measured directly, whereas consumption rates were estimated from a model by using gut fullnesses. Second, consumption estimates are directly dependent on temperature, an independent variable in these analyses. Finally, we were interested in determining the conditions that stimulate feeding, which we believe are more immediately reflected in the gut fullness measures.

Feeding patterns at the individual level were investigated by examining the relationships between gut fullness and the independent variables of body size, lipid level, water temperature, and time of year. Gut fullness values of individual fish were dominated by zeros (empty stomachs) and could not be transformed appropriately, preventing the use of parametric statistics. Because empty stomachs may reflect either a lack of prey availability or reduced appetite, we concentrated analyses at the individual level on the slope of the 95% quantile of gut fullness regressed against the independent variables (Scharf et al., 1998). This technique has been used to examine scatter-plots, when boundaries of a relationship between two factors are of interest, rather than the mean. In our case we were interested in the factors that stimulate feeding as opposed to developing a predictive relationship between independent variables and gut fullness. The 95% quantile line describes the relationship between maximum observed gut fullness and the independent variables. Mean gut fullness of all fish captured on a given date was used to investigate feeding patterns at the population level and was compared to average lipid level of fish captured on that date, time of year, and water temperature, by using Kendall's coefficient of rank correlation (Sokal and Rohlf, 1981).

Lipid levels were determined for a subsample of fish from the diet analysis. This involved determination of the percentage of dry body weight comprising non polar lipids (those used primarily for energy storage). Details of the compositional analysis procedure can be found in Hurst et al. (2000) and Schultz and Conover (1997). Compositional analysis was performed on 15 to 40 fish from each sampling date (except 13 December 1995). The relationship between lipid level and gut fullness at the individual level was examined by using all fish for which both pieces of information were available (n=587), whereas analyses at the population level used the average lipid level observed on a given date (n=28).

Results

Gastric evacuation rate

Evacuation rates of overwintering YOY striped bass declined greatly with temperature. At mid-winter temperatures, evacuation rates were among the lowest reported for any fish species. Time to 50% evacuation ranged from 31 hours at 11°C to 101 hours at 2°C (Fig. 1). The best fit parameters in the evacuation model were b_0=–0.00685, b_1=0.126, c_0=25.757, and c_1=–1.8033.

We observed a lag between feeding and a measurable loss of material from the stomach. The length of the observed lag decreased as temperature increased from 18.2 hours at 2°C to 6.1 hours at 11°C. The exponential models used here to describe digestion fitted the experimental data as well as, or significantly better than, other common models (linear and square root; Bromley 1994).

The amount of variability among individuals in evacuation rate increased as temperature decreased, leading to a poorer fit of the evacuation models at the lower temperatures (Fig. 1). Deviations from the model increased significantly as temperature decreased (P=0.018; ANOVA of absolute value of sample deviations from evacuation model). Body size had no effect on evacuation rate among juvenile striped bass. We found no correlation between residual values from the evacuation model and fish length (r=–0.03 P=0.685).

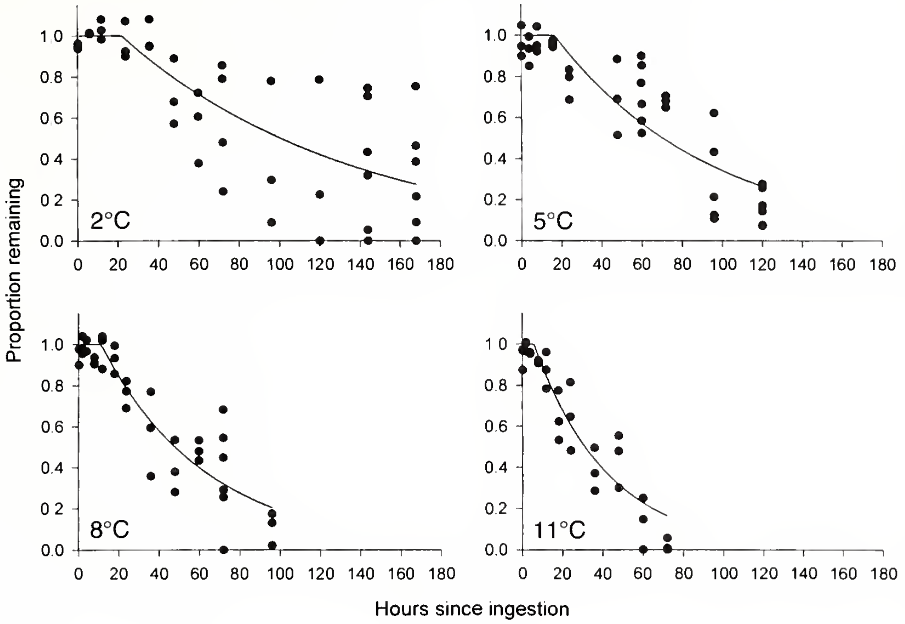

Figure 1

Gastric evacuation patterns of YOY striped bass fed *Crangon septemspinosa* at four temperatures. Points are observations of percentage of initial meal remaining in the stomach after a predetermined time. Lines represent evacuation model fitted by using all data.

Diet composition

Benthic invertebrates were the dominant prey of YOY striped bass overwintering in the lower Hudson River estuary, making up 95.0% of the diet by weight; the remaining 5.0% were various fish prey (Table 1). Most striped bass captured during winter had empty stomachs (64.8%), and on two dates, all fish sampled had empty stomachs (30 March 1994 and 6 December 1994). Unidentifiable stomach contents ranged from 6.0% weight (%W) in 1995 to 29.2%W in 1996. The most common item in the diet was gammarid amphipods (those identified to genus were *Gammarus* sp., but most were not identified) making up 51.3%W and occurring in 81% of stomachs with food (%F). Several species of shrimp were also common dietary items, including sand shrimp (*C. septemspinosa*) and grass shrimps (*Palaemonetes* spp.). Other invertebrates that were important items on several dates included mysid shrimp, polychaetes, and oligochaetes. Fish were rarely found in the stomachs of YOY striped bass (3.8%F) but included bay anchovy (*Anchoa mitchilli*), Atlantic silversides (*Menidia menidia*), and American sand lance (*Ammodytes americanus*).

The importance of individual prey taxa varied among years and among dates within each year, although low sample sizes of fish with prey prevent drawing strong conclusions from these data. In 1994, striped bass fed almost exclusively on gammarid amphipods (90.2%W and 92.1%F), but these prey were less important in other years. Shrimp were important in 1993 (33.5%W, mostly *C. septemspinosa*) and 1995–97 (34.9 to 43.6%W all species). Polychaetes were observed in striped bass diets only in 1995 (23.3%W and 17.9%F). Oligochaetes were found commonly in 1997, averaging 18.5%F and 23.9%W of the diet but did not occur in other years.

Consumption rates

Assuming an exponential evacuation pattern, consumption rate estimates for overwintering YOY striped bass ranged from 0 to 0.29% body weight per day (%bw/day, Fig. 2). Standard deviations of estimated consumption averaged 46% of the estimate (range: 20.2–100.9%). The standard deviation of the consumption estimate was relatively insensitive to assumptions of the covariance between $\overline{S}$ and R_e. The ratio of estimates under the least conservative assumption (perfect covariance) to that under the most conservative assumption (perfect negative covariance) was less than 1.08, with one exception (1.17 on 20 December 1993). The standard deviations presented in Figure 2 are the intermediate values, based on the assumption of no covariance.

Feeding patterns

Among individuals, we observed a strong negative relationship between maximum gut fullness and the level of storage lipids. The 95th quantile of gut fullness was 3% for fish with lipid reserves of 2% dry weight and decreased to under 1% for fish with lipid levels in excess of 19% dry weight (Fig. 3). No significant patterns were observed between individual gut fullness levels and water temperature, time of year, or body size. At the population level, only date was significantly correlated with mean gut fullness (Kendall's rank correlation: $P=0.032$; Fig. 4). Addition of temperature and average lipid level did not provide significant improvements in the fit of the model ($P>0.20$ for both).

Discussion

Evacuation rates

Laboratory-measured evacuation rates of YOY striped bass at representative winter temperatures were among the lowest reported for any fish species. None of the estimates compiled from multiple studies by He and Wurtsbaugh (1993) included rates as low as the 0.027 we measured at 11°C. The reason for this observation is not that striped bass have exceedingly low digestion rates compared with other fishes, but rather that evacuation rate is strongly related to temperature and is rarely measured at the lower end of temperatures encountered by each species. For example, the lowest temperature at which evacuation rates in white perch (*Morone americana*) have been measured is 12.6°C (Parrish and Margraf 1990), despite the fact that this fish often occupies waters near 0°C. The only evacuation rates we found in the literature comparable to those we measured were those for largemouth bass at 4°C (R_e=0.006; Cochran and Adelman, 1982, from data in Markus, 1932) and for brown trout at 1.8°C (R_e=0.026; Jensen and Berg, 1993), both measured near the species' thermal minima.

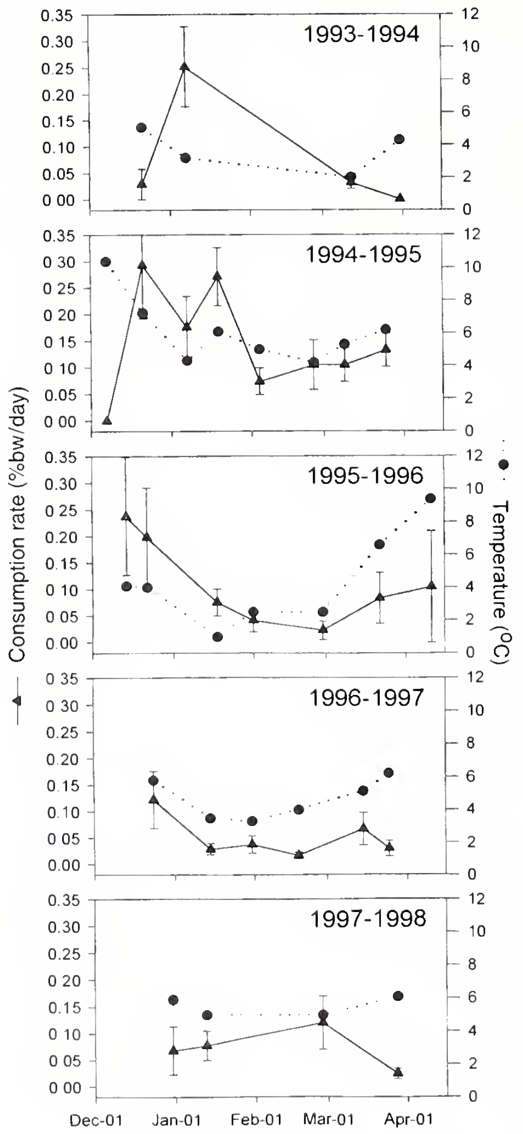

Figure 2

Consumption estimates (%body weight/day [±1 SD]) for YOY striped bass in the Hudson River estuary through five winters, and bottom water temperatures observed during sampling.

Factors other than temperature have been found to influence evacuation rates such as prey type, method of feeding (voluntary vs. force-feeding), and body size. All of our experiments were conducted with a single prey type (*C. septemspinosa*) at one ration level (2% of body weight). Meal size has been found to affect evacuation rate in laboratory studies (Smith et al., 1989; Andersen, 1998), but this effect is substantially smaller than that of temperature (He and Wurtsbaugh, 1993). Our meal size was slightly higher than the mean, but well within the range observed among individual fish. We found that body size did not affect evacuation rate over the size range of overwintering YOY striped bass (88–150 mm). In several studies comparing evacuation rates of various prey, large bodied shrimp, including *C. septemspinosa*, were found to be evacuated at lower rates than soft bodied prey or smaller shrimp species (Nelson and Ross, 1995; Singh-Renton and Bromley, 1996; Lankford and Targett, 1997), whereas other studies found no differences among prey types (Juanes and Conover, 1994; dos Santos and Jobling, 1995). Although *C. septemspinosa* and other shrimp species are common diet items of YOY striped bass, they were not the dominant item. If other prey items, such as amphipods, are digested and evacuated more rapidly, consumption rates of wild fish will be underestimated when non-shrimp prey dominate the diet.

We allowed a lag in our description of meal evacuation based on laboratory observations. Such a lag has been observed in several other studies, including both those where fish fed voluntarily (Gerald, 1973; Grove et al., 1985) and were force-fed (Vondracek, 1987). The length of the lag observed in juvenile striped bass decreased as temperature increased, as seen in juvenile turbot (*Scopthalmus maximus*; Grove et al., 1985). The lag prior to beginning of evacuation could be an artifact of experimental conditions or a natural delay in the passing of food from the stomach to the intestine following ingestion of shelled prey. Our model estimated the evacuation rate parameter (R_e) after the lag because this parameter represents the rate of passage of food from the stomach. Consumption rates based on gut fullness levels may be overestimated if there is a considerable lag prior to the beginning of digestion. However, the digestive lag we observed was short compared with the total evacuation time, averaging only 11% of the time required to reach 75% digestion. Simulations that varied the length of the digestive lag time and the evacuation rate showed that for lag times in the range we observed, consumption rates were overestimated by less than 5%. If the lag observed in the laboratory experiments does not occur in the wild, estimated consumption rates are unbiased.

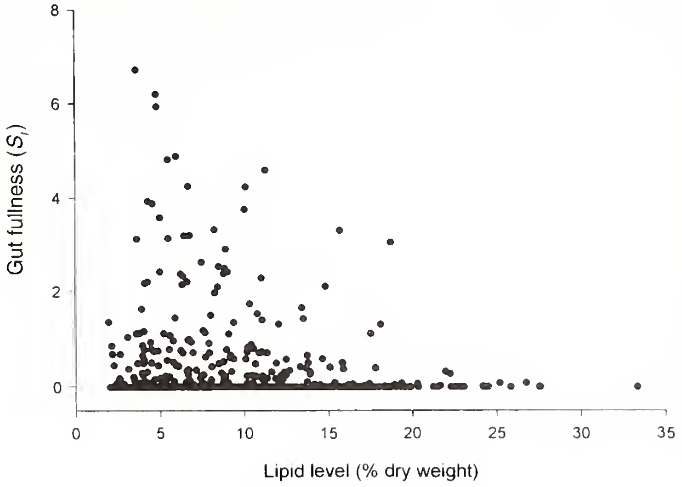

Figure 3

Relationship between gut fullness and lipid level of overwintering YOY striped bass through five winters. Points are gut fullness and lipid levels of individual fish.

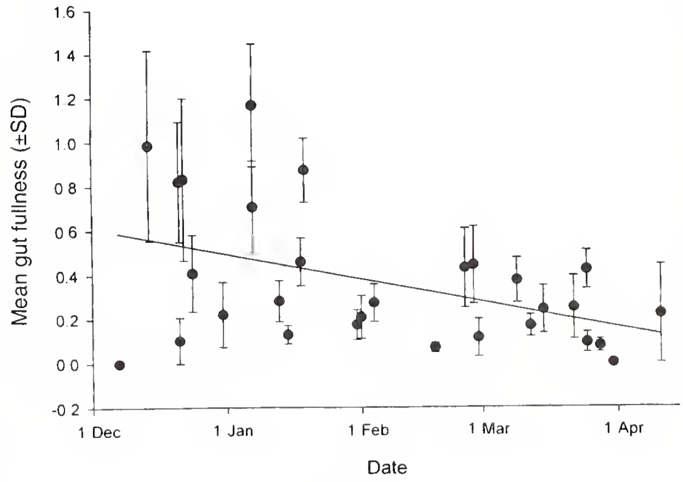

Figure 4

Relationship between mean gut fullness (±1 SE) observed on a given date and date for collections of YOY striped bass through five winters.

Diet

Diets of overwintering YOY striped bass in the lower Hudson River estuary were dominated by benthic invertebrates such as gammarid amphipods and several shrimp species. Juvenile striped bass do not appear to undergo a major diet shift from summer to winter but appear to focus more heavily on amphipods in winter. Studies of summer diets of striped bass in the Hudson River from the 1970s and 1990s found a slightly more diverse diet than we observed in winter. Summer diets included copepods, chironomids, and isopods, and more commonly incorporated fish and polychaetes (Gardinier and Hoff, 1982; Hurst, unpubl. data). Data from other estuaries suggest that the diets of overwintering juvenile striped bass may vary regionally. In Chesapeake Bay, Hartman and Brandt (1995a) found that fish prey accounted for 20–25%W of YOY striped bass diets in winter, substantially more than the 2–9%W we observed in the Hudson River. In the Miramichi River estuary, overwintering fish fed primarily on shrimp (mysids and *C. septemspinosa*) and ceased feeding when temperatures fell below 3°C in late November (Robichaud-LeBlanc et al., 1997). The studies from Chesapeake Bay and the Miramichi River estuary did not examine interannual variability in diets.

Overwintering juvenile striped bass appear to be opportunistic feeders, their diets reflective of the epibenthic invertebrate community in the lower Hudson River and similar to published information on the diets of co-occurring species. Although there have been no surveys of the benthic community in the lower Hudson River in winter, data available from summer surveys in the Hudson River estuary and the adjacent Raritan estuary suggest dominance of the epibenthic community by gammarid amphipods, shrimp, and annelids (Ristich et al., 1977; Steimle and Caracciolo-Ward, 1989). The winter fish community in the lower Hudson River is composed primarily of striped bass, white perch, Atlantic tomcod (*Microgadus tomcod*), and winter flounder (*Pseudopleuronectes americanus*). Winter diets of Atlantic tomcod were dominated by gammarid amphipods and copepods and were significantly less diverse in winter than in spring and autumn (Grabe, 1977, 1980). Diets of age-1 and older striped bass overwintering in the lower Hudson River estuary are similar to those of YOY fish, although the incidence of juvenile fish prey (including YOY striped bass) increased with body size (Dunning et al., 1997).

Consumption rates

Consumption estimates of overwintering YOY striped bass in the Hudson River were consistently below 1%bw/day, with only 34% of captured fish containing prey items. Our field estimates of the consumption rates of overwintering striped bass are significantly lower than published estimates of maximum consumption measured in laboratory experiments (Hartman and Brandt, 1995b). Their estimates of maximum consumption (for a 10-g fish) increased from 0.45%bw/day at 1°C to 7.10%bw/day at 10°C. The highest percentages of predicted maximum consumption achieved by YOY striped bass in the Hudson River occurred on 6 January 1994 (25.8%), and on three dates in early winter 1995 (15.5–18.0%). On other dates consumption was generally below 10% of maximum estimated from the Hartman and Brandt (1995b) model.

Bull et al. (1996) developed a model of consumption for overwintering juvenile Atlantic salmon in which appetite was related to anticipated metabolic requirements. To minimize the risks of starvation and predation associated with foraging, they predicted that appetite of overwintering fish should be highest in early winter (when future metabolic needs are greatest) or when internal energy reserves are low. Our results suggest that a similar model might be appropriate for overwintering striped bass. Gut fullness levels of YOY striped bass were related to level of lipid reserves at the individual level (Fig. 3). Although some fish had empty stomachs at all energy levels, the highest observed gut fullnesses were found in fish with low lipid levels. This finding suggests that overwintering striped bass increase feeding activity when energy reserves become depleted. Such feeding patterns have been documented in laboratory experiments with Atlantic salmon (Metcalfe and Thorpe, 1992) and striped bass (Hurst and Conover, 2001) but have not previously been observed among fish feeding in the wild.

At the population level, we observed a negative relationship between mean gut fullness and date; gut fullnesses were higher in early winter than late winter (Fig. 4). This pattern was predicted in the Atlantic salmon model (Bull et al., 1996) but could also be due to external factors such as depleted food resources at the end of winter. Benthic prey production is likely reduced by low winter temperatures, and standing stocks may become depleted as winter progresses. Further work is required to determine fully the causes and implications of reduced gut fullnesses observed in later winter. Reduced feeding in late winter may reflect the availability of sufficient energetic reserves and suggests that starvation is unlikely. Conversely, reduced feeding due to depressed prey availability in late winter would indicate a strong potential for winter starvation. The role of starvation in winter mortality will depend greatly on determining if variable feeding patterns among years are due to internally controlled variations in feeding motivation or environmentally imposed constraints on prey availability.

Acknowledgments

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Use of a continuous egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*) off California

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Abstract—In 1996, using the continuous underway fish egg sampler (CUFES), we carried out a preliminary study with the daily egg production method (DEPM) for estimating fish biomass of Pacific sardine, *Sardinops sagax*. Full-water-column abundance of sardine eggs was correlated with the abundance of eggs taken at 3-m depth with a CUFES; however direct conversion of CUFES samples to the full-water-column abundance, required by the DEPM, would add considerable variance to an estimate of daily egg production. Our preliminary study also indicated that the average size of an egg patch for Pacific sardine was 22 km diameter and that all stages of sardine eggs were not equally vulnerable to a CUFES at 3-m depth.

Using these findings as a guide, in 1997 we carried out an adaptive allocation DEPM survey in which the numbers of sardine eggs collected with the CUFES were used to determine subsequent locations of vertical net tows. All the vertical net tows were taken in a high-density stratum where the CUFES collected at least two eggs per minute; egg density in this stratum was computed by using only vertical tows. The remaining survey area, where the CUFES collected fewer than two eggs per minute, constituted a low-density stratum where egg density was estimated by using the ratio of CUFES egg abundance in these two strata multiplied by the egg density in the high-density stratum. Conventional statistics were used because the sampling units were survey lines spaced at 22-km or greater intervals. An acceptable level of precision (CV=21%) for a daily production of 2.57 eggs 0.05 m^2/d (51.4 eggs m^2/d), was achieved by using only 141 vertical net tows. Therefore, the CUFES will enhance the DEPM by increasing precision of the estimates, or by reducing costs in relation to a survey composed of only vertical water-column tows, when the CUFES is used adaptively to establish sampling strata for vertical water column tows.

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The continuous underway fish egg sampler (CUFES) (Checkley et al., 1997; van der Lingen et al., 1998; Checkley et al., 1999; Watson et al., 1999) is a new device that provides high-resolution spatial maps of fish eggs by sieving the eggs from water pumped from a fixed depth while a survey ship is underway. These data may be used as an index of fish abundance, or to study spawning habitats, and if converted to the numbers of eggs in the full-water-column, they may be used to estimate fish biomass by using one of the egg production methods (Hunter and Lo, 1997).

The objective of our study was to evaluate the use of the CUFES in the daily egg production method (DEPM) of estimating the spawning biomass of pelagic fishes (Lasker et al., 1985; Parker, 1985). In the DEPM, biomass is calculated from the number of staged eggs taken in plankton samples and the daily fecundity of the parents. In a standard DEPM estimate, eggs are sampled in vertical net tows, starting from a point below the maximum depth of the eggs (typically 70 m) and ending at the surface. These vertical samples are taken within a grid of stations located 4 nmi apart, a sampling interval known to produce uncorrelated samples of anchovy eggs (Smith and Hewitt, 1985). Use of the CUFES in the DEPM has several potential advantages over the use of a standard fixed-grid survey. Continuous sampling may increase the precision of the biomass estimate because it provides an increased spatial resolution of egg patches (Hunter and Lo, 1997). Continuous sampling may save ship time and thereby reduce the cost of sampling per transect mile, and the increased spatial resolution of egg patches provides new knowledge regarding the spawning behavior of the species. Several potential disadvantages of using a CUFES in the DEPM also exist: the gains in precision provided by continuous sampling may be diminished because of the increases in variance due to converting numbers of eggs taken in a CUFES to a full-water-column abundance; a much more complicated formula for variance of the estimate may be needed because of correlated CUFES samples; and the numbers of staged eggs taken in a CUFES may be biased, either because a CUFES damages eggs, making the staging of them more subject to error or because all stages may not be equally vulnerable to sampling at the 3-m depth. Clearly these issues need to be resolved if a CUFES is to be used in a DEPM survey.

We used the following approach to evaluate the use of a CUFES in the estimation of daily egg production. We conducted a pilot CUFES survey in 1996 with the objective of examining the spatial properties of Pacific sardine (*Sardinops sagax*) eggs sampled by a CUFES. We determined how the numbers of eggs taken in a CUFES were related to their abundance in the full-water-column, the spatial correlation of egg samples, and the condition of eggs after passing through a CUFES. Our

Table 1

Total number of samples and positive samples of sardine eggs sampled in CUFES and CalVET surveys. The range and mean of duration (minutes) for CUFES collections for 1994, 1996, and 1997 surveys.

		1996 pilot CUFES survey		1997 DEPM survey	
	1994 (18 Apr—11 May)	leg 1 (15–21 Mar)	leg 2 (21 Mar–6 Apr)	leg 1 (11 Mar–27 Mar)	leg 2 (28 Mar–7 Apr)
CUFES[1] (positive)	—	1396 (889)	905 (568)	896 (550)	331 (137)
Duration mean range (min)	—	3.5 2–5	18.13 3–35	30 1–54	26.9 1–34
CalVET[2] (positive)	684 (74)	91 (66)	— —	141 (102)	— —
Survey area (km^2)	380,175 (253,850 in USA)		157,000	174,196	

[1] Total collection in leg 1, 1996, was 1437. The first 41 tows were experimental.

[2] CalVET surveys in 1996 and 1997 were taken in the high-density stratum; in 1994 they were taken in a fixed grid over the whole survey area.

analysis of the 1996 cruise was used to develop an adaptive allocation survey design, similar to that proposed by Thompson et al. (1992), to estimate the biomass of sardine that incorporates a CUFES into the DEPM. Using the adaptive allocation survey design, we conducted a DEPM survey for Pacific sardine with a CUFES in 1997, and the precision of our estimate of daily production of eggs was compared with the precision of the estimate provided by a DEPM survey carried out in 1994 under standard methods (Lo et al., 1996). We also considered the accuracy of shipboard counts of eggs collected in a CUFES in relation to counts of preserved samples made after cruise end. We present our results in chronological order, beginning with those from the 1996 pilot survey which provided the rational for the new survey design in 1997; we next describe the new survey design, and end with a comparison of the 1997 CUFES and DEPM survey with the conventional DEPM survey in 1994.

Survey data and spatial models

Survey data

The data used in our study were taken from three ichthyoplankton surveys (Table 1):

1 A pilot CUFES cruise in 1996. This cruise consisted of leg 1 (during which both a CUFES was used and full-water-column tows were taken) and leg 2 (during which only a CUFES was used to survey the large geographic area of spawning sardine (Fig. 1);
2 A DEPM survey in 1997. This survey for Pacific sardine employed a new survey design with the CUFES and the California vertical tow (CalVET, see below) (Smith et al., 1985) (Fig. 2). The allocation of CalVETs was determined by the egg density observed from the CUFES.
3 Results of the 1994 DEPM survey off California, U.S., and Baja California, Mexico (Lo et al., 1996). This was a conventional fixed-grid DEPM survey employing only CalVETs. This cruise was used as a standard for comparing DEPM surveys with and without the CUFES.

The CalVET net consisted of 150-µm nylon netting. The diameter of the CalVET net frame was 25 cm; the tow was lowered to a depth of 70 m and was retrieved vertically. The CUFES was installed midship on the NOAA vessel *David Starr Jordan* onto the intake pipe over the side of the vessel; it extended 3 m below the water surface (see illustration in Checkley et al., 1997). Eggs were sieved from the water flow with the 500-µm nylon mesh of the CUFES concentrator.

The density of eggs taken in the CalVET net was expressed as the number of eggs/0.05 m^2 of sea surface water, a standard procedure in the DEPM, where 0.05 m^2 is the area of the mouth opening of the CalVET net. All eggs taken in the CalVET samples, regardless of survey, were counted and staged in the laboratory. The density of eggs taken in the CUFES was expressed as the number of eggs taken per minute. The interval over which eggs accumulated in CUFES samples varied depending on their abundance. When abundance was low, samples were collected over intervals of 0.5 h equivalent to 7.4 km or 4 nmi on the transect line; when abundance was high, they were collected over intervals of at least 1 minute (0.24 km or 0.13 nmi). All CUFES samples were counted at sea, preserved, and recounted in the laboratory. All eggs taken by the CUFES in the pilot survey, but not the subsequent DEPM survey, were staged in the laboratory. We used the system detailed in Lo et al. (1996) and grouped eggs by their ages into half-day age classes for egg mortality computation and one-day age classes for spatial statistical analysis.

In our study, we considered the estimates of only the daily production of eggs, P_0, one of the key parameters in

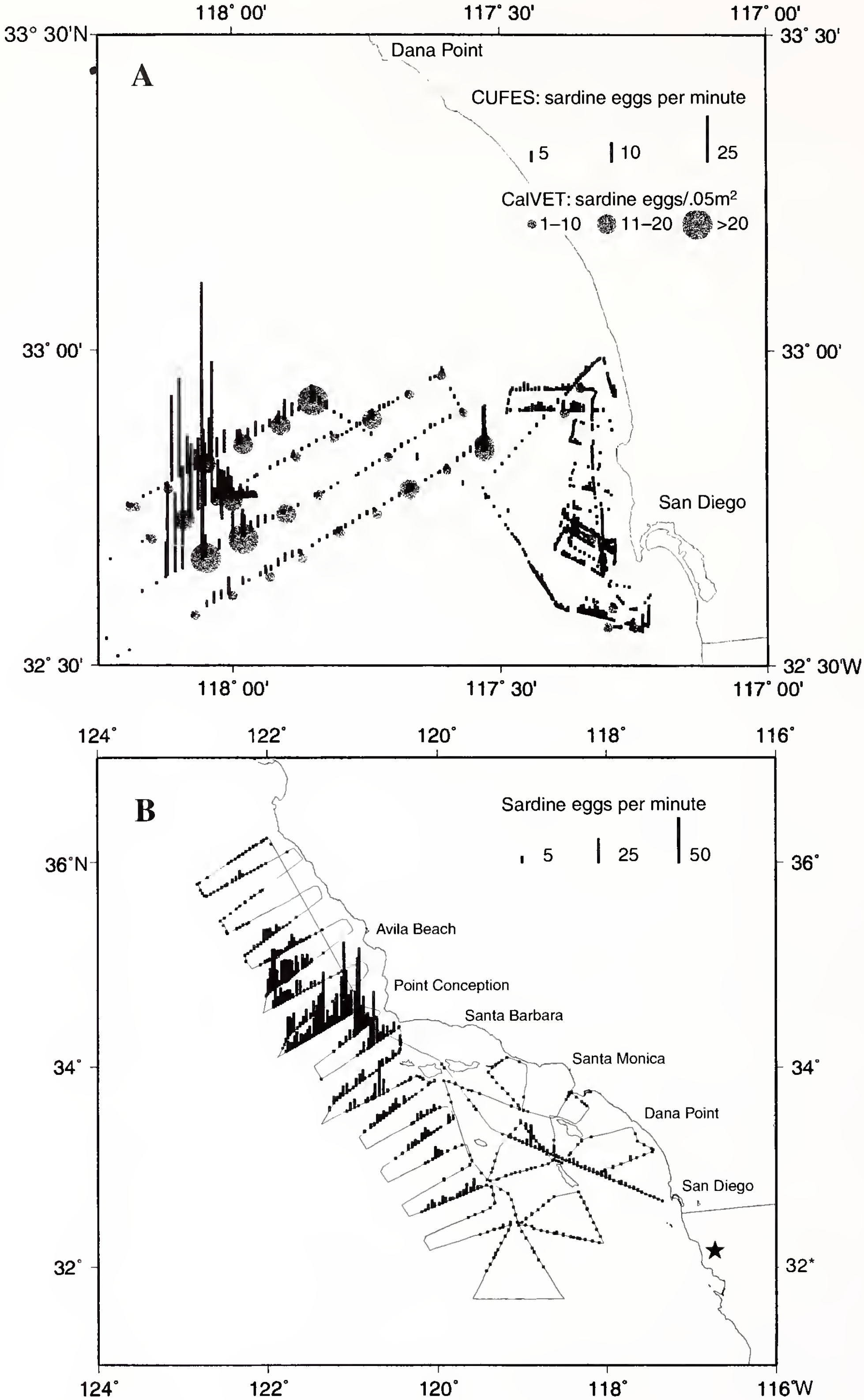
A
Dana Point
CUFES: sardine eggs per minute
5
10
25
CalVET: sardine eggs/.05m²
1–10
11–20
>20
San Diego
33° 30'N
33° 00'
32° 30'
33° 30'
33° 00'
32° 30'W
118° 00'
117° 30'
117° 00'
B
Sardine eggs per minute
5
25
50
Avila Beach
Point Conception
Santa Barbara
Santa Monica
Dana Point
San Diego
36°N
34°
32°
36°
34°
32°
124°
122°
120°
118°
116°
116°W

Figure 1

Sardine eggs/minute from CUFES samples and survey pattern in cruise 9603. Leg 1: 13–21 March 1996 (**A**) and leg 2 (**B**): 6–21 March 1996. The star on the lower right corner of section B is the reference point (0,0) to compute variograms (see text).

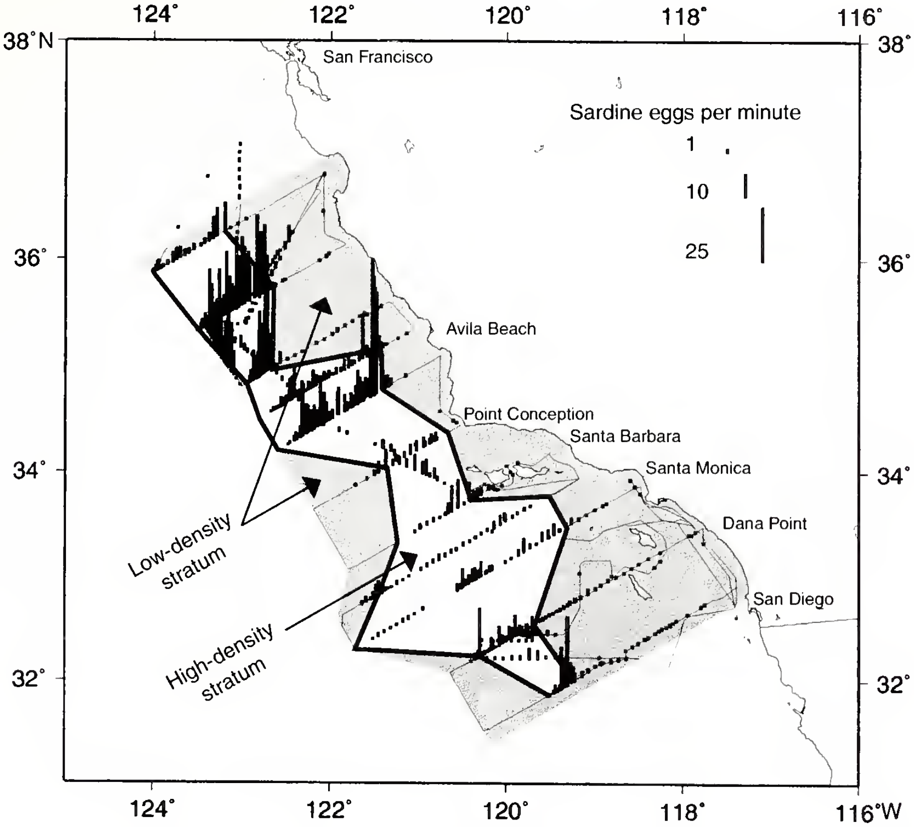

Figure 2

Sardine eggs/minute from CUFES samples and survey pattern in cruise 9703, 11 March–6 April 1997, with two strata: a high-density stratum (open area) and a low-density stratum (shaded area).

the DEPM. P_0 is only one of six parameters used in estimating biomass with the DEPM. In the DEPM, biomass is related to egg production using the model $B_s = P_0 A / (R / W_f) SF$, where B_s = biomass for area A; P_0 = the daily egg production at age 0 day per unit sea surface area; W_f = the average female weight; S = the fraction of females spawning per day; F =batch fecundity; and R = the fraction of the biomass that is female (Alheit, 1993; Hunter and Lo, 1997). The denominator, $(R / W_f)SF$, is the number of eggs/biomass in grams and is also called the daily specific fecundity. In the 1994 survey all parameters were estimated but in the 1997 survey we estimated only P_0 and A and used historical data for the other parameters (Hill et al.[1]).

Variograms of sardine density (eggs/minute)

As the first step in developing a DEPM survey design for the CUFES, we used geostatistical techniques (Cressie, 1991; Barange and Hampton, 1997; and Fletcher and Sumner, 1999) to describe the spatial structure of the egg distribution and estimated the major diameters of sardines egg patches from the 1996 survey. We applied variogram models (Cressie, 1991; Petitgas, 1993) to CUFE samples (in units of eggs/minute) grouped into three age groups: 1-day (4–27 h), 2-day (28–51 h), and 3-day (52–75 h).

Variogram ($\gamma(h)$) is defined as the variance of difference between values that are h units apart and is a function of variance and covariance:

$$2\gamma(h) = \mathrm{var}\left[u(x) - u(x+h)\right] \qquad (1)$$
$$= 2\left(\mathrm{var}(u) - \mathrm{cov}(h)\right) \qquad \text{if } \mathrm{var}\left[u(x)\right] = \mathrm{var}\left[u(x+h)\right],$$

where $u(x)$ = the eggs/min at location x;
$u(x+h)$ = the eggs/min at the location h (nmi) away from x;
$\mathrm{var}(u)$ = the variance; and
$\mathrm{cov}(h)$ = the covariance of eggs/min that are h (nmi) apart.

$\gamma(h)$ is the semivariogram. For simplicity, we refer to $\gamma(h)$ as the variogram. We used S+SpatialStats (Kaluzny et al.,

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[1] Hill, Kevin, T. M. Yaremko, L. D. Jacobson, N. C. H. Lo, and D. A. Hannan. 1998. Stock assessment and management recommendations for Pacific sardine (*Sardinops sagax*) in 1997. Marine region, Admin. Rep. 98-5, Cal. Dep. of Fish and Game, 8604 La Jolla Shores Dr., La Jolla, CA 92037.

1996) and EVA software (Petitgas and Prampant[2]) to analyze and visualize spatial distributions of sardine eggs/min and to compute a variogram for each of the three age groups of sardine eggs. Two basic assumptions of the variogram (Eq. 1) are intrinsic stationarity and isotropy. Intrinsic stationarity means that a constant mean exists and the variance of egg density is defined by the magnitude of h. Isotropy means that spatial correlation and the range of correlation do not change with direction. The variogram is normally expressed as a function of three parameters: range, sill, and nugget effect. The range is the distance beyond which the observations are not correlated. The sill is the variance of the random field and is the asymptotic value of $\gamma(h)$. The nugget effect measures the micromeasurement error and the white noise for h close to 0 (Cressie, 1991).

Ideally, for h close to 0, the variogram ($\gamma(h)$) will be close to zero because the observations tend to be similar. As h increases, the observations become h units apart and tend to be different, or cov(h) decreases, and the variogram increases. At a certain distance, h^*, cov(h) approaches zero, and for $h > h^*$, the variogram approaches its asymptote (sill). The distance, h^*, is the range. The range (h^*) was estimated from a model that best fits the data and was used to estimate the diameter of the patch of sardine eggs because eggs whose distances are less than h^* nmi are correlated and thus are likely to be in the same patch. Conversely, eggs that are more than h^* nmi apart are no longer correlated and thus are assumed to be in different patches. We chose the robust (or stable) estimator of the variogram (Cressie and Howkins, 1980; Cressie, 1991):

$$2\hat{\gamma}(h) = \frac{\left[\sum_{N(h)} \left|u(x) - u(x+h)\right|^{1/2}\right]^4}{\left[0.457 + 0.494/\left|N(h)\right|\right] N(h)^4}, \qquad (2)$$

where $|N(h)|$ = the number of distinctive pairs; and
h = the distance (nmi) between any two locations.

To avoid possible trends, we first ran a local regression model (LOESS) of ln(eggs/min+1) against line distance (the distance computed from the survey lines, y-axis) and station distance (x-axis) (Chamber and Hastie, 1992) where the reference point (0,0) is a pseudo station in Mexico (station 260 on CalCOFI line 980; Fig. 1B). We then constructed a variogram for the residuals from the local regression model (LOESS) in four directions clockwise from the transect (0° ,45°, 90°, and 135°) to examine possible anisotrophy. We chose natural logarithm (ln) transformed data because the distribution of eggs was skewed. Finally, we used an interactive S+ function (model.variogram function) to determine the estimates of parameters for each variogram: range, sill and nugget effect. The range was then used as the estimate of the diameter of the patch for each of the three age groups and total number of eggs.

Results of the 1996 pilot CUFES survey

Spatial correlation and patch size of sardine eggs

For each of the three age groups and the total number of eggs, the four-directional variograms of the residuals of ln(eggs/min+1) from LOESS (Fig. 3) indicated that the variograms for transects at 0°, 45°, and 135° were clearer than the cross-transect (90°), particularly for the 1-day-old eggs, 2-day-old eggs, and total egg category. The variogram in the direction of within-transect (0 degree) had the clearest signal because intervals between adjacent collections were the shortest. Because the variograms for each direction looked similar, we used the variogram in the within-transect direction to assess the spatial correlation of sardine eggs.

The spherical model was chosen to fit the variogram (Cressie, 1991):

$$\gamma(h;\theta) = \begin{cases} 0 & h = 0 \\ c_0 + c_s\left[(3/2)\left(\|h\|/a_s\right) - (1/2)\left(\|h\|/a_s\right)^3\right] & 0 < \|h\| < a_s \\ C_0 + C_s, & \|h\| \geq a_s. \end{cases} \qquad (3)$$

where c_0 = the nugget effect;
c_s = the practical sill (variance – nugget);
a_s = the range; and
$\| h \|$ = Euclidean distance.

For total number of eggs (all eggs combined), the range of the residuals of ln(eggs/min+1) was 22.2 km (12 nmi), sill (variance in the random field) was 0.4, and nugget was 0.05. For 1-day-old eggs, the range was 14.8 km (8 nmi) and the sill was 0.3. For 2-day-old eggs, the range was 18.5 km (10 nmi), the sill was 0.15, and nugget was 0.005, and for 3-day-old eggs, range was 22.2 km (12 nmi), the sill was 0.065, and nugget was 0.005 (Fig. 4). Because the maximum range was 22.2 km (12 nmi), eggs collected more than 22.2 km (12 nmi) apart were considered uncorrelated; therefore, transect lines spaced intervals of 12 nmi or greater were considered independent. The data also indicated the gradual dispersion of egg sardine patches with time as described by Smith (1973) because the diameter of sardine egg patches increased from 14.8 km for 4–27 h old eggs to 22.2 km for eggs 52–75 h old.

Conversion of CUFES egg density to full-water-column abundance and distribution of egg stages

Egg counts from 91 paired samples collected with the CUFES and CalVETs during leg 1 of the1996 survey (Table 1) were used to derive a conversion factor from eggs/minute of CUFES sample to CalVET catch (R). We used a regression estimator to compute the ratio of eggs/minute from the CUFES to eggs/tows from CalVETs, $R = \mu_y/\mu_x$, where y = eggs/minute; x = eggs/tow; and R = the catch ratio. The estimator of R is $\hat{R} = \Sigma(x \times y)/\Sigma(x^2)$.

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[2] Petitgas, P., and A. Prampant. 1993. EVA (estimation variance), a geostatistical software on IBM-PC for structure characterization and variance computation. CM1993/D:65, 81st meeting of ICES. IFREMER laboratoire d'Ecologie Halieutique, BP 21105,44311 Nantes (France), Cedex 03, 33 p.

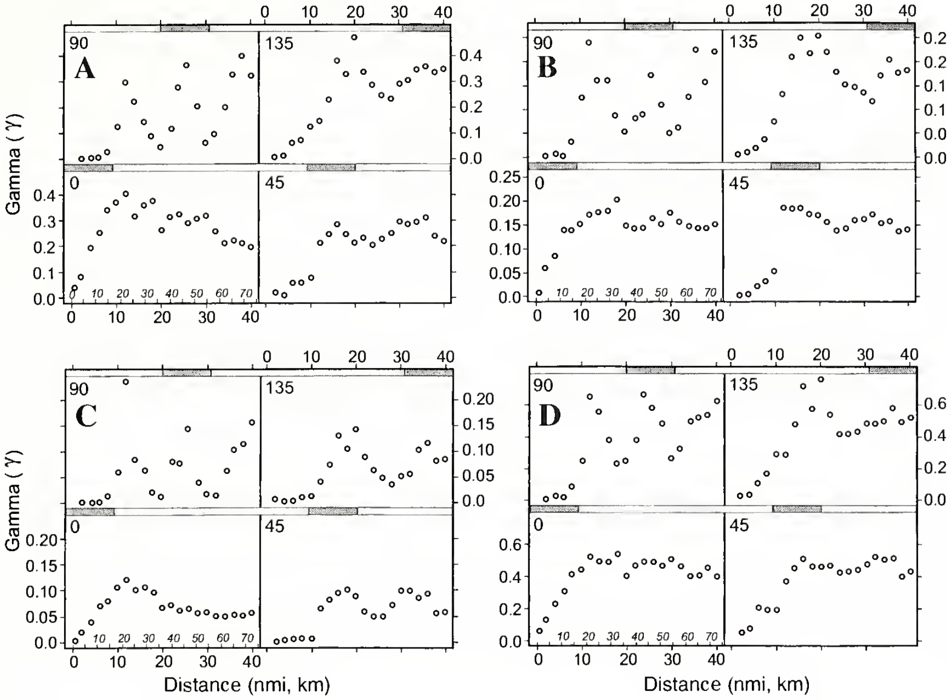

Figure 3

Variogram of residuals of ln (*sardine eggs*/minute+1) for age groups: 4–27 h (**A**), 28–51 h (**B**), and 52–75 h (**C**), and total eggs for four directions (**D**). Degree 0 is the direction perpendicular to the coastal line, degree 90 is the direction along the coastal line during leg 2 of cruise 9603. On the x-axis, the inner ticks are in km and the outer ticks are in nmi.

Paired samples were taken wherever high abundance of eggs appeared in samples collected with the CUFES. We obtained egg/minute = 0.73 eggs/0.05m^2 (CV=0.16) (Fig. 5). This means that for one egg observed from a CalVET, one would expect to see, on average, 0.73 eggs/min. Or for one egg/min from a CUFES, one would expect to see 1.5 eggs/tow. A striking difference existed between the data from the 1996 pilot survey and the full survey carried out in 1997. In 1997, the catch ratio of eggs/minute to eggs/tows was 0.25 (CV=0.08) from 110 pairs of CalVET and CUFES of which at least one sample was positive (Fig. 5). This means that one egg/tow from a CalVET tow was equivalent to approximately 0.25 egg/min from a CUFES, or one egg/minute from the CUFES was equivalent to 4 eggs/tow from a CalVET sample. The ephemeral nature of such conversion coefficients was not known to us when we developed the design for the 1997 survey. However, the variance associated with the direct 1996 conversions was a strong incentive to reduce the effects of direct conversions in the design of the 1997 survey and in the calculation of biomass.

To determine if the CUFES provides an unbiased sample of all sardine eggs stages, we compared the distributions of developmental stages between the two samplers taken in 91 paired CUFES and CalVET samples during leg 1 of the 1996 survey (Fig. 1). A 2 × 11 contingency table was constructed for total counts of each of 11 stages of eggs collected with the CalVET and the CUFES. A chi-square statistic was computed to test the null hypothesis that the distribution of stages was independent of the samplers.

The chi-square (χ^2) analysis showed that the distribution of stages was not the same between the two samplers (χ^2=188.47, df =10, P-value <0.01) (Table 2). The difference was primarily due to eggs of stages I, III, V, and VI. The CUFES caught only two stage-I eggs; therefore we decided to run a χ^2 test with stage-I and stage-II eggs collapsed into one group. A similar conclusion was reached for the later case (χ^2=160.31, df =9, P-value <0.01). A large χ^2 value indicates that staged sardine eggs in the upper 3 m may not be representative of the sardine egg stages in the whole-water-column and that the egg production computed from the CUFES survey would be biased.

Design for the 1997 CUFES and DEPM survey

The foregoing analysis of the 1996 survey data indicated that adequate correlation exists in overall egg abundance between the CUFES and the full-water-column egg samples, but a direct conversion of CUFES data to full-water-column abundance would add considerable variance to an estimate of daily egg production. The foregoing analysis

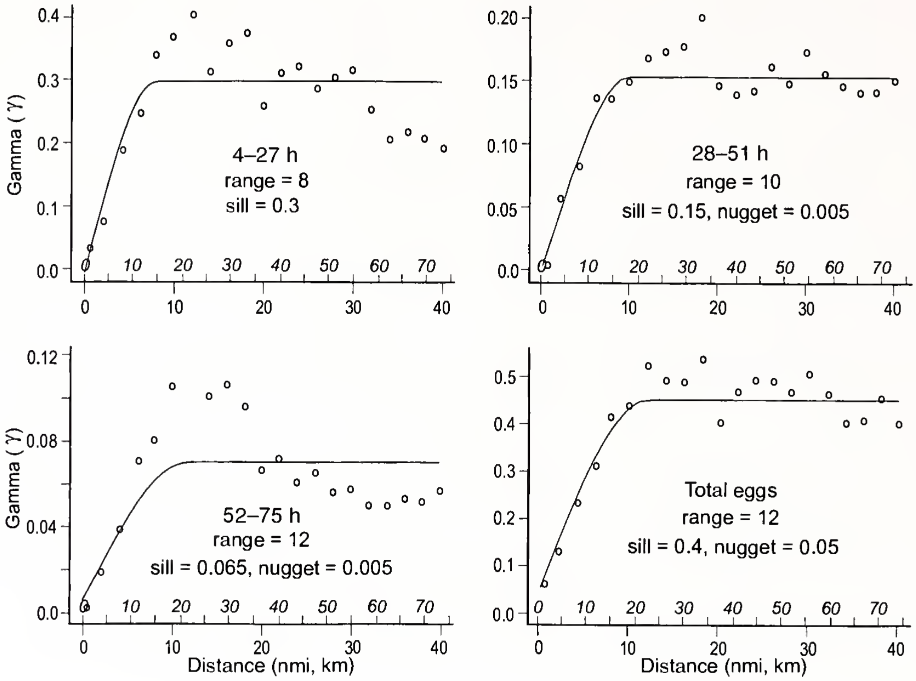

Figure 4

Spherical model fitted to variograms of residuals of ln (*sardine eggs*/minute+1) for three age groups and total eggs, in the direction perpendicular to the coastal line during leg 2 of cruise 9603. On the x-axis, the inner ticks are in km and the outer ticks are in nmi.

Table 2

Contingency table of sardine egg counts by developmental stages and two samplers: the CalVET and the CUFES, leg 1, 1996.

	Egg stages										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Total count											
CalVET	31	63	116	46	36	95	23	27	37	15	13
CUFES	2	74	466	44	148	104	103	22	75	21	14
Percent											
CalVET	6	13	23	9	7	9	5	5	7	3	3
CUFES	0	7	43	4	14	10	10	2	7	2	1

also indicated that a potential bias may exist if staged CUFES eggs are used to estimate daily egg production (P_0) because all stages of eggs may not be equally vulnerable to the CUFES. A further complication was found, in that egg samples taken in the CUFES were strongly correlated up to distances of 22.2 km, necessitating the use of geostatistics if individual samples were to be used for the estimates. We concluded from these findings that the most effective way to use the CUFES in the DEPM design was to use it to allocate full-water-column tows with a CalVET. This adaptive allocation design, similar to one proposed by Thompson et al. (1992), takes advantage of the rapid continuous monitoring capability of a CUFES, while preserving the quantitative features of the CalVET.

We decided to allocate a CalVET at 4-nmi intervals, whenever catch from the CUFES was greater than or equal to 2 eggs/min[1] When the catch from the CUFES began to decrease to fewer than 2 eggs/min, then CalVETs were stopped (Fig. 6). This critical value for the allocation of CalVETs was equivalent to slightly more than three eggs per CalVET tow and was based on the conversion factor from the pilot study of CUFES eggs/min = CalVET 0.73 eggs/tow. The CUFES was in operation continuously along each transect line, and samples were taken at an interval

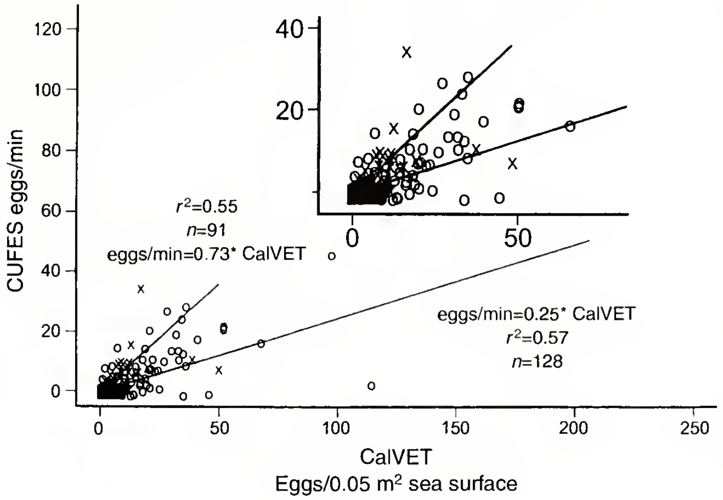

Figure 5

Conversion coefficient: CUFES eggs/min = CalVET 0.73 eggs/tow in 1996 and CUFES eggs/min = CalVET 0.25 eggs/tow in 1997. Insert is an expansion of the graph near the origin. x = data obtained from 1996; o = data obtained from 1997.

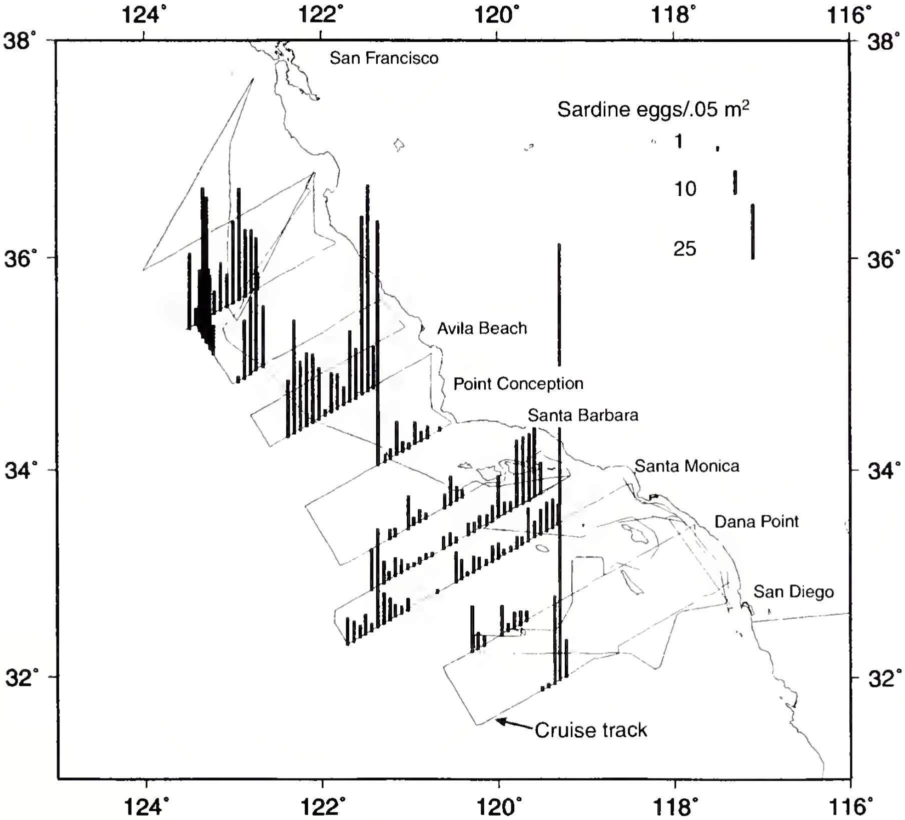
124°
122°
120°
118°
116°
38°
36°
34°
32°
San Francisco
Sardine eggs/.05 m^2
1
10
25
Avila Beach
Point Conception
Santa Barbara
Santa Monica
Dana Point
San Diego
Cruise track

Figure 6

Sardine eggs/0.05 m^2 from CalVET samples in cruise 9703 survey,11 March–7 April 1997. Lines (perpendicular to the coast) are the cruise tracks.

ranging from 1 to 54 minutes with a mean of 30 minutes and median of 15 minutes (Table 1). Only samples from CalVETs were used to model the sardine embryonic mortality curve, thereby avoiding a possible stage-specific bias with the CUFES.

Spacing of transect lines was also determined to some extent by samples from the CUFES. The scheduled spacing of lines was 40 nmi apart but if a high density of sardine eggs was encountered, an additional transect line was added between the two scheduled lines. As a result of this procedure, two lines were added (lines 4 and 8) in the 1997 DEPM survey (Fig. 2).

To estimate P_0, the survey area was then stratified into two strata: a high-density stratum where the density of eggs taken by the CUFES was at least 2 eggs/min; and a low-density stratum (Fig. 2) where only CalVET samples were taken and where the density of eggs was less than 2 eggs per minute. Within each stratum, the transect line was selected as the sampling unit both for CalVET samples and for CUFES samples. Because lines were spaced at intervals greater than 12 nmi, statistical procedures based on uncorrelated data were used (correlation should not be ignored if the distances are less than 12 nmi). We describe below the two different estimation methods used to estimate P_0: one for the high-density stratum and the other for the low-density stratum; we combined the two methods to estimate P_0 combined for the total survey area.

We used transects as the primary sampling units and applied conventional statistical methods to construct the mortality curve from CalVET samples (Armstrong, 1988). CalVETs, the secondary sampling units, were taken for the first ten transects, except transect number eight. The P_0 was estimated for each of two strata by using different methods, and an weighted average was obtained for the whole survey.

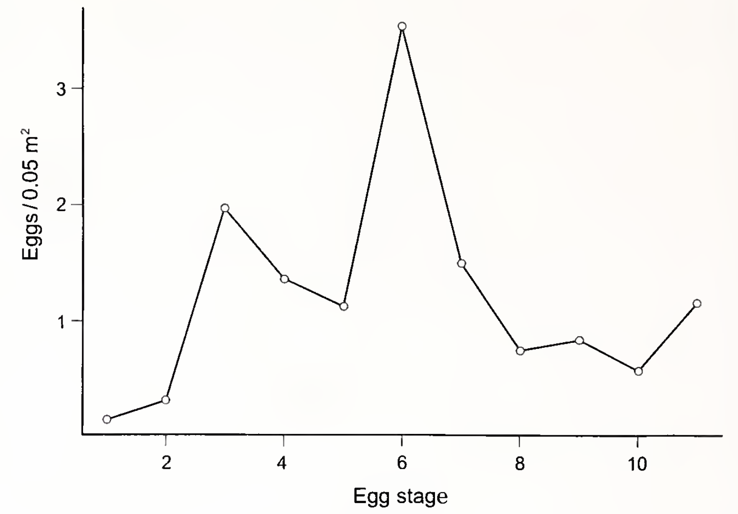

Figure 7

Sardine eggs/0.05 m² for each developmental stage in the high-density stratum, cruise 9703, 11 March–7 April 1997.

Table 3

Daily sardine egg and yolksac larval production and their standard error in the high-density stratum in 1997.

	Age (day)	Daily production/ 0.05 m²	Standard error
Eggs	0.32	3.29	1.32
	0.76	4.48	2.10
	1.17	4.32	2.05
	1.71	6.12	2.88
	1.92	3.21	1.60
	2.52	1.86	0.66
Yolksac larvae	5.62	0.65	0.16

Egg production in the high-density stratum ($P_{0,1}$)

Ages of staged sardine eggs (Fig. 7) were assigned according to a temperature-dependent time-to-stage model (Lo et al., 1996). Sardine eggs were grouped in half-day categories excluding those eggs that were newly spawned during the first three hours. An average eggs-per-tow and an average age were obtained for each half-day category within each transect. A weighted average number of eggs in each half-day category was obtained where the weight is duration of the CUFES sampling interval for each transect line (Eq. 4, Table 3).

$$P_t = \frac{\sum_i P_{it} m_i}{\sum_i m_i} \qquad \operatorname{var}(P_t) = \frac{n/(n-1)\sum_i m_i^2 (P_{it} - P_t)^2}{\left(\sum_i m_i\right)^2}. \qquad (4)$$

where P_{it} = the eggs or yolksac larvae/0.05 m²; and
m_i = the total CUFES sampling time (minutes) for the ith transect for $i = 1, \ldots, 7, 9$, and 10.

For a weighted nonlinear regression, the weight is $1/\mathrm{SE}(p_t)$.

The yolksac larval stage includes larvae from time of hatching to the time they form functional jaws, i.e. larvae ≤6 mm live length or 5 mm captured length (Zweifel and Lasker, 1976). As with the egg data, the average number of yolksac larvae per tow was calculated for each transect and the overall mean catch of yolksac larvae was computed as a weighted average. Yolksac larva production was the mean catch of larvae ≤5.00 mmi (captured size) per CalVET divided by its temperature-dependent stage duration (Zweifer and Lasker, 1976). The age of yolksac larvae was estimated by the average of minimum age and maximum age, i.e. hatching time + age of forming functional jaw)/2 (Lo et al., 1996) (Table 3).

With these data, an embryonic mortality was modeled by an exponential curve (Eq. 5) (Lo et al., 1996). The mortality curve was fitted to eggs/0.05 m^2 in each age class and numbers of yolksac larvae/0.05 m^2 with an assigned age based on observed mean water temperature. The estimates of the intercept ($P_{0,1}$) and the instantaneous mortality rate from the mortality curve were obtained by a nonlinear regression of S+ nonlinear regression function (nls()).

The exponential embryonic mortality curve is

$$P_t = P_{0,1}\exp(-zt), \qquad (5)$$

where P_t = a weighted average of eggs – yolksac larvae /0.05m^2; and

t = the mean age (d) for each of 6 half-day age groups of eggs and yolksac larvae. The weights are total CUFES sampling time (minutes) for each transect (Eq. 4).

Daily egg production in the low-density stratum ($P_{0,2}$)

Because no CalVET samples were taken in the low-density stratum, we estimated daily egg production ($P_{0,2}$) in that stratum as the product of the egg production in the high-density stratum ($P_{0,1}$) and the ratio (q) of egg/min in the low-density stratum to that in the high density stratum from the CUFES samples. Here we assumed that q is the same no matter whether it was computed from eggs/min by the CUFES or from eggs/tow by the CalVET:

$$P_{0,2} = P_{0,1}\, q, \qquad (6)$$

$$q = \frac{\sum_i \frac{\bar{x}_{2,i}}{\bar{x}_{1,i}} m_i}{\sum_i m_i}, \qquad (7)$$

where m_i = the total CUFES time (minutes) in the ith transect; and

x_{ji} = was eggs/min in the jth stratum and ith transect.

The variance of q was computed according to that of a ratio estimator (Eq. 4).

Daily egg production for the total survey area (P_0)

P_0 was computed as a weighted average of $P_{0,1}$ and $P_{0,2}$, where

$$\begin{aligned} P_0 &= \frac{P_{0,1}A_1 + P_{0,2}A_2}{A_1 + A_2} \qquad (8)\\ &= P_{0,1}w_1 + P_{0,2}w_2 \\ &= P_{0,1}[w_1 + qw_2] \end{aligned}$$

and according to Goodman (1960), the unbiased estimate of var(P_0) is

$$v(P_0) = v(P_{0,1})(w_1 + w_2 q)^2 + P_{0,1}^2 w_2^2 v(q) - v(P_{0,1}) w_2^2 v(q), \qquad (9)$$

where $w_i = \frac{A_i}{A_1 + A_2}$, $i = 1,2$, and A_i = the area size.

Simulation

Bootstrap simulations were conducted to provide the possible biases and another estimate of the standard error of daily egg production (P_0) and the instantaneous mortality rate (z) for each stratum and the entire survey area under the adaptive allocation sampling scheme. As mentioned earlier, CalVETs were taken on nine transect lines and not on line 8 and line 11. In the simulation, nine transects with CalVETs were sampled with replacement and estimation procedures described in previous section were followed. To evaluate the effect of weighting, we included weighted and unweighted nonlinear regression where the weight was the inverse of the standard error of egg production of each age group and yolksac larvae. One thousand iterations were run, and the standard deviation of 1000 estimates was the bootstrapped standard error of the estimates. Bias was the difference between the average of 1000 estimates and the estimate from the original data. The bias-corrected estimate was the original estimate minus the bias.

Results of the 1997 CUFES and DEPM survey

Daily egg production

The daily egg production for each half-day category and yolksac larval production and their ages (d) were used to construct an embryonic mortality curve for the high-density stratum (Eq. 5, Fig. 8, Table 3). The daily egg production in the high-density stratum ($P_{0,1}$) based on unweighted nonlinear regression was 5.04 eggs/0.05 m^2/d (100.8 eggs/m^2/d ,CV=0.25) and egg mortality was z=0.21 (CV=0.73) for an area (A_1) of 66,841 km^2 (19,530 nmi^2) (Eq. 8, Fig. 9). The ratio (q) of egg density between the low-density stratum and high-density stratum from CUFES samples was 0.211 (CV=0.43) (Eq. 7). Therefore, in the low-density stratum, the egg production ($P_{0,2}$) was 1.064 eggs/0.05 m^2/d (21.28 eggs/m^2/d, CV=0.49) for an area (A_2) of 107,255 km^2 (31,338 nmi^2). The estimate of the daily egg production for the entire survey area was 2.57/0.05 m^2 (51.4/m^2, CV=0.27) (Eq. 8, Table 4). The weighted nonlinear regression produced estimates slightly different from those with unweighted nonlinear regression: $P_{0,1}$ =4.76/0.05 m^2 (59.2/m^2, CV=0.18), z = 0.35(CV=0.14), and the P_0 for the entire survey area was 2.43/0.05 m^2 (48.6/m^2, CV=0.21).

The bootstrap estimate of P_0 (5.10) was similar to the original estimate P_0 (5.04) in the high-density stratum. The standard error of P_0 (1.6) from the bootstrap analysis was higher than the estimate from the original data (1.28). The bias of P_0 (0.06) was negligible because the ratio

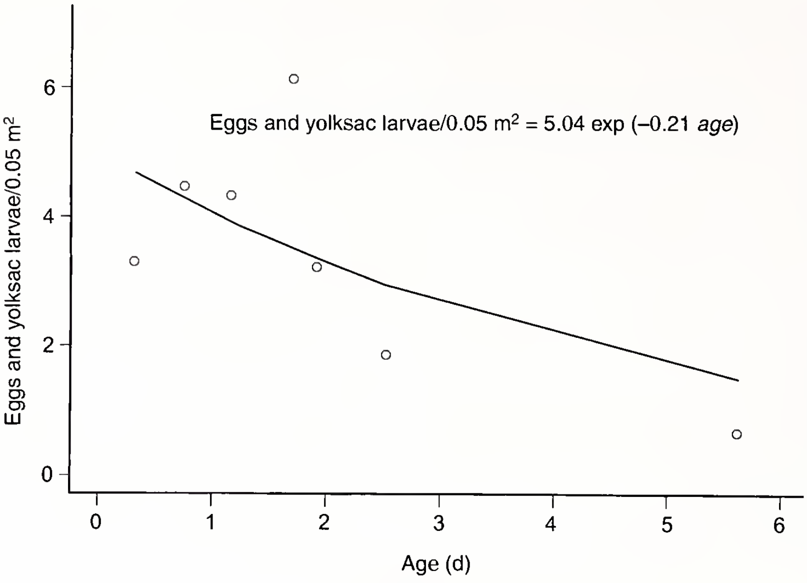

Figure 8

Embryonic mortality curve of sardine eggs and yolksac larvae in the high-density stratum, 9703. The last data point represents yolksac larvae (Table 3).

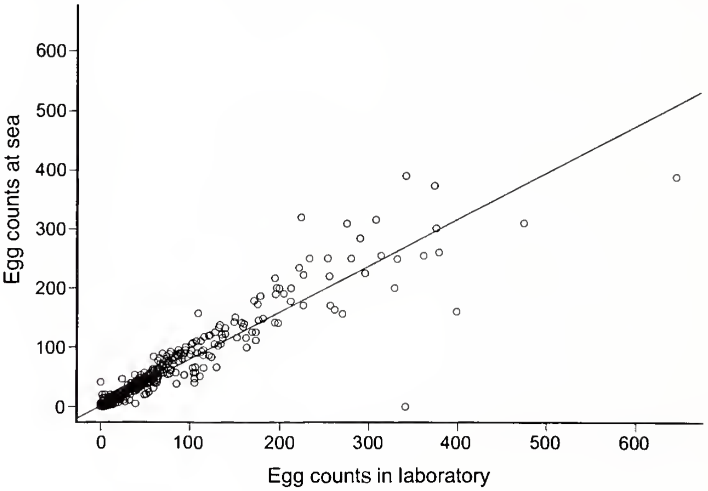

Figure 9

Relationship between egg counts at sea and those taken in the laboratory from CUFES samples, cruise 9703. The diagonal line is the fitted regression line: egg counts at sea = 0.8 egg counts in laboratory.

of bias to standard error was less than 0.25 (Efron and Tibshirani, 1993). However, the daily instantaneous mortality rate (z=0.29) from the bootstrap was higher than 0.21 from the original data. The bias-corrected z was 0.13 (SE=0.12). For the entire survey area, the bootstrapped P_0 was 2.60/0.05 m^2 (SE=0.71; CV=0.27), similar to the original estimate (2.57/0.05 m^2).

The bootstrap results indicated that the weighted nonlinear regression produced a downward biased estimate of P_0 (bias=–1.26=3.50–4.76) in the high-density stratum. The standard error (1.60) of P_0 from bootstrap was also higher than that computed from the original data (0.86). The fact that the ratio of bias to standard error (1.26:1.60) was greater than 0.25 may indicate that the weighted non-

Table 4

Estimates of daily egg production (P_0: number of eggs/0.05 m^2/day at age 0), daily instantaneous mortality rate (z) for the high-density stratum (66,841 km^2) and low-density stratum (107,255 km^2), P_0 for the entire survey area (174,096 km^2), the catch ratio of eggs/min in the low-density stratum to eggs/min in the high-density stratum (q), their standard errors (SE), estimated bias, and the bias-corrected estimates for the unweighted and weighted nonlinear regression from 1000 iterations of bootstrap simulation.

	High-density stratum				Low-density stratum		Entire survey area			
	$P_{0,1}$	SE ($P_{0,1}$)	z	SE (z)	$P_{0,2}$	SE ($P_{0,2}$)	P_0	SE (P_0)	q	SE (q)
Unweighted nonlinear regression										
survey	5.04	1.28	–0.21	0.16	1.06	0.52	2.57	0.72	0.211	0.09
bootstrap										
mean	5.10	1.60	–0.29	0.28	1.10	0.53	2.60	0.85	0.22	0.08
SE	1.60	0.72	0.12	0.11	0.48	0.28	0.71	0.34	0.09	0.03
CV	0.31	0.46	–0.40	0.38	0.45	0.52	0.27	0.41	0.41	0.40
bias	0.06	0.32	–0.08	0.12	0.03	0.01	0.03	0.13	0.009	–0.01
bias corrected	4.98	0.96	–0.13	0.04	1.04	0.51	2.54	0.58	0.202	0.10
Weighted nonlinear regression										
survey	4.76	0.86	–0.35	0.050	1.004	0.45	2.43	0.51	0.211	0.09
bootstrap										
mean	3.50	0.72	–0.27	0.056	0.730	0.33	1.80	0.41	0.22	0.08
SE	1.60	0.35	0.10	0.025	0.400	0.21	0.76	0.20	0.09	0.03
CV	0.46	0.49	–0.36	0.440	0.550	0.63	0.42	0.48	0.42	0.40
bias	–1.26	–0.14	0.08	0.006	–0.274	–0.12	–0.63	–0.10	0.009	0.01
bias corrected	6.02	1.00	–0.43	0.044	1.278	0.57	3.06	0.61	0.202	0.10

linear regression was unwarranted in our case. One possible reason is that the standard errors (SE) of egg production in each age group were between 1.3 and 2.8, whereas the SE for yolksac larvae was 0.16 (Table 3). Although the weighted regression should be used when the variances of data are unequal, we believe that there is too large a disparity between variance of eggs and yolksac larvae, and too much weight was assigned to yolksac larvae for a weighted nonlinear regression.

The spawning biomass was computed from the daily egg production from the 1997 survey, and the historical daily specific fecundity (number of eggs/gram of biomass) was 23.55 (Macewicz et al., 1996) assuming the daily specific fecundity was the same as that for 1994–96. Sardine spawning biomass in 1997 would be 379,940 t[3] for an area of 174,096 km^2 (50,868 nmi^2) from San Diego to San Francisco. No variance of B_s was computed because no variance of the number of eggs per population weight (g)/day was available.

Comparison of results from the 1997 CUFES and DEPM survey with results from a conventional DEPM survey

We compared the results of the 1997 CUFES and DEPM design with those of a conventional DEPM (1994), in which only CalVET samples were taken, to illustrate how the CUFES allocation design may affect the performance of a DEPM survey of Pacific sardine. We believe the comparison is instructive, even though the two surveys differed somewhat in area and population size; the conventional 1994 survey covered a larger area than the 1997 survey (380,175 km^2 vs. 174,096 km^2), and, the total biomass of sardine was smaller in 1994 than in 1997 (111,493 t (Lo et. al., 1996) vs. 379,940 t, respectively). In both surveys staged eggs and yolksac larvae from CalVET samples were used in the calculation of P_0.

An obvious difference in the results of the two surveys was that only 11% (74/684) of CalVET samples were positive for sardine eggs in the 1994 conventional survey, whereas in the 1997 survey 72% (102/141) were positive (Table 5). These results indicate that CUFES was effective in allocating CalVET samples and thereby reducing ship-time costs per survey mile. The coefficients of variation (CV) for the estimates of P_0 were similar: 0.22 for the conventional survey compared with 0.27 for the CUFES-based DEPM. Thus, variance penalty for using the ratio estimator q did not greatly diminish the benefit in using the CUFES in the DEPM. This simple statistical comparison, however, does not reveal the greatest potential benefits in using a CUFES. The allocation of CalVETs would be most useful when the population is at a lower level, as it was in 1994, because at such levels one must cover a large survey area to assure an unbiased estimate, but the population is probably concentrated in a very small fraction of the area where CalVET samples will be allocated. In addition, the high-resolution maps of the spatial distribution

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[3] The spawning biomass would be 359,280 metric tons if P_0 of 2.43/0.05 m^2 from weighted nonlinear regression was used.

Table 5

Sardine daily egg production (P_0) from a conventional survey (1994), compared with a CUFES and DEPM survey (1997).

1994 Conventional DEPM survey Area: 380,175 km^2		1997 CUFES and DEPM survey Area: 174,096 km^2	
CalVET samples		CalVET samples	
total	684	total	141
positive for eggs	72	positive for eggs	102
positive percent	11%	percent positive	72%
CUFES samples	None	CUFES samples	
		total	1227
		total positive	687
		percent positive	56%
		high-density stratum	84%
		low-density stratum	40%
Daily egg production		Daily egg production	
P_0	0.169/0.05 m^2	P_0	2.57/0.05 m^2
CV	0.22	CV	0.27
spawning biomass	111,493 t	spawning biomass	379,940 t

of eggs provided by the CUFES have not as yet been incorporated into the DEPM design, but we believe in the long-term it will be possible to improve the accuracy of surveys by doing so, as well as possible to develope new insights into the processes involved in selection of spawning habitats by parent fish.

Comparison of shipboard egg counts with preserved egg counts

A key element of the allocation design was that the allocation of CalVET samples was based on near real-time counts and on the identification of eggs by CUFES operators. After having been counted by CUFES operators at sea, the eggs were preserved in vials, and later recounted and identified in the laboratory by experts. We compared egg counts in the laboratory with those taken at sea from the 1997 survey to determine the reliability of shipboard counting and identification and to indicate the extent to which a difference affected the final estimate of the daily egg production.

Within each stratum, we first computed an overall mean eggs/min for the laboratory and ship count by using a ratio estimator (where y=total number of eggs and x=the total minutes for each transect summed over all transects) (Table 6). We also computed a ratio of eggs/min from the laboratory to that from the ship for each transect line and obtained a weighted ratio for each stratum, where weight was the duration for each transect within a stratum (Eq. 7). The ratio for the entire survey area was a weighted average of two ratios, one from each stratum, and weight was the area of each stratum, as in Equation 5.

In the high-density stratum, the ratio from the laboratory counts to the ship counts was 1.19:1 (CV=0.03). In the low-density stratum, the ratio from the laboratory to the ship was 1.22:1 (CV=0.05) (Table 6). The overall ratio for the entire survey area was 1.20:1 (CV=0.10); therefore the laboratory count was higher than the ship count by 20% (Table 6).

Of a total 1227 collections, 687 were positive according to laboratory counts. There were 16 collections where ship counts were positive but counts in the laboratory were zero. Eggs of other species in those 16 CUFES collections were obviously misidentified as sardine eggs. (Table 6). A total of 658 pairs had positive counts, out of which 130 pairs had equal positive counts. A total of 524 pairs had zero counts. Therefore there were 654 equal counts between laboratory and ship (524 zeros and 130 positive counts) and 573 pairs (=1227–654) with a mismatch. The relationship between the total counts from the laboratory and the ship confirmed the undercount from the ship (Fig. 9). The variance of undercounts increased with the total counts from the laboratory. One outlier was a collection where the ship count was zero and the laboratory count was 341. Although the absolute undercounts increased with the total number of eggs, the percent of undercount decreased with the total egg count.

Conversion coefficient between two strata (q)

In the 1997 CUFES and DEPM survey, one of the primary functions of the CUFES collections was to provide a conversion coefficient (q) of egg density between two strata to convert the egg production in the high-density stratum ($P_{0,1}$) to the low-density stratum ($P_{0,2}$), i.e. $P_{0,2} = P_{0,1} \times q$ (Eq. 6). The conversion coefficients (q) computed from the laboratory and ship counts were similar: 0.213 (CV=0.44) from the laboratory and 0.211 (CV=0.43) from the ship counts. Therefore, the bias of using egg counts at sea to calibrate egg production per unit area in the low-density stratum would be small: if the ratio of 0.213:1 were used, the egg production for the entire survey area would be 2.60/0.05 m^2 instead of 2.57/0.05 m.^{2}

Table 6

Comparison of egg density and distribution of zero and positive egg counts from CUFES samples with those taken in the laboratory and at sea (ship), 1997. CVs are in shown in parentheses.

Egg density (eggs/min)	High-density stratum	Low-density stratum	Overall	q
ship	4.16 (0.41)	0.47 (0.45)		0.213 (0.44)
laboratory	4.91 (0.4)	0.57 (0.45)		0.211 (0.43)
ratio	1.19 (0.03)	1.22 (0.05)	1.20 (0.10)	

Distribution of zero and positive egg counts

	Laboratory		
Ship	zero	positive	total
zero	524	29	553
positive	16	658[1]	674
total	540	687	1,227

[1] 658 = 130 with exact match + 428 without match.

Discussion

Presently the most productive use of the CUFES in the DEPM is to use the CUFES as an efficient auxiliary information provider and the CalVET, or another full-water-column tow, as the primary sampler. Given our present level of knowledge of egg distributions and accuracy of predicting them, it would be folly to depend only on the CUFES for an estimate of P_0. Predictive egg-distribution models (Sundby, 1983; Westgard, 1989) are promising and sometime in the future might make exclusive use of the CUFES practical in a DEPM. Exclusive use of the CUFES as a sampler in the DEPM also would require detailed knowledge of the stage-specific vulnerability of eggs to the sampler because a bias seemed to exist for sardine. In short, full-water-column tows are essential for accurately estimating egg production today, but the CUFES can greatly facilitate the allocation of such tows.

The CUFES enhancement of the basic DEPM design (Lasker, 1985) may have broad application because the DEPM is used world-wide for estimating the spawning biomass of sardines, anchovies, and other species of fishes (Priede and Watson, 1993; Zeldis, 1993; Lo, 1997). Thus, we feel it is useful to discuss some of the new features of the DEPM survey that we developed for estimating P_0 with the CUFES.

Critical value for allocation sampling

To use the CUFES in our DEPM survey design requires selecting an egg density that triggers full-water-column sampling (CalVET sampling). We used a critical value of 2 eggs/min, which was equivalent to 3 or 8 eggs/tow, depending on the conversion factor. If the 1997 conversion factor (eggs/min=0.25 egg/tow) was correct, we could lower the critical value to 1 egg/min, equivalent to 4 eggs/tow. This would create a larger high-density stratum and more CalVET tows would be allocated. This range of critical values (3–8 eggs/tow) was similar to the value (5 eggs/tow) used in a stratified sampling design for an anchovy survey in Biscay Bay in Spain (Petitgas, 1997). As a result, the precision of P_0 and z would likely be improved. Increasing the area for the high-density sampling also reduces the potential for bias from the assumption of a constant egg mortality between strata. On the other hand, lowering the critical value diminishes the gain from using the CUFES. Clearly, an optimal critical value exists for each species and survey area. The critical value can be determined prior to the survey or during the survey by using order statistics (Thompson and Seber, 1996; Quinn et al., 1999).

The extent that the critical value can be fine tuned to deliver an optimum balance between the CUFES and CalVET samples for a particular region, species, and season is unknown. The large difference in catch ratios between our 1996 (0.73) and 1997 (0.25) surveys certainly does not support the idea of fine tuning. These differences may overstate the expected variability for sardine because the areas were different; the 1996 samples were taken over a very limited portion of the survey area , whereas in 1997, the sample pairs were taken where high-density spawning occurred (Figs. 1A, 2, and 6). Interestingly, our 1997 estimate (0.25) is similar to that computed by us for sardine off South Africa (van der Lingen et al., 1998).

Variability in catch ratios

The extent of vertical mixing of eggs is probably the main factor affecting the variation of the catch ratio between surveys. Because the selection of the optimal critical value for CalVET sample allocation depends upon on the relationship between catches in the CUFES and those in the CalVET, or the catch ratio, it seems useful to consider what the ratio would be if water were perfectly mixed in the whole-water-column. If the water were perfectly mixed, fish eggs would be distributed randomly and the catch ratio (between CalVET and CUFES) would be a constant, because egg count would be proportional to the volume of water filtered through the two samplers. A CUFES filters on the average 0.64 m^3 of water/min and the CalVET filters 3.5 m^3 of water. Therefore under perfect mixing, the ratio of eggs/min to eggs/tow = 0.64 m^3/3.5 m^3 = 0.18 for the daytime when fish schools are in deeper water, assuming the vertical distribution of sardine eggs was similar to that of anchovy eggs (Moser and Pommeranz, 1999). At night, when fish schools are in the upper 50 meters,[4] the ratio would be 0.64 m^3/2.5m^3 = 0.26. This means that on the average, for four to six eggs seen in a CalVET catch, we would expect one egg/min in the CUFES. The fact that the estimated ratio for two years were 0.76 in 1996 and 0.25 in 1997, indicates that more sardine eggs appeared in the upper 3 m than would be expected under perfect mixing and less mixing occurred in 1996 than in 1997. Clearly, if all the eggs were in the upper 3 m of water column, the ratio would be 1:1 for the same surface area. As the extent of vertical mixing in the sea is highly variable, we believe that calibration tows are always needed, even if the CUFES is used only as an index of egg abundance. Vertical egg mixing models might eventually help to reduce calibration requirements.

Identification and counting of eggs at sea

Identification and counting of fish eggs while the ship is underway was an essential ingredient of our adaptive allocation design. The eggs of some commercially important clupeid species are often difficult to distinguish from those of co-occurring species (Ahlstrom and Moser, 1980; Matarese and Sandknop, 1984; Watson and Sandknop, 1996). Further, many melanostomiin species have eggs with characteristics (e.g. large diameter, wide perivitelline space, segmented yolk) that are similar to those of co-occurring sardines and other clupeid eggs. During a DEPM survey off Oregon, Bentley et al. (1996) encountered a type of melanostomiin egg similar to the egg of Pacific sardine at 24 of 46 stations. Our experience has shown that the risk of misidentification increases when identifications are made at sea; during our CUFES and DEPM survey in 1997, about 1% of the eggs initially identified as sardines turned out to be melanostomiin eggs after examination in the laboratory. Fortunately, melanostomiin eggs were in such low abundance that they had no effect on the critical values of density. The possibility of misidentification differs depending on season, location, and target species. Clearly all shipboard positive records for areas that lie outside of the known spawning range and season of the target species should be checked in the laboratory after the cruise. Owing to the great abundance of sardines and anchovy eggs, the effect of misidentification on biomass estimation is probably trivial if the survey is conducted during peak spawning months.

Egg counts on shipboard were somewhat lower than shoreside counts. Even though the effect of the difference is negligible from the standpoint of biomass estimation, we recommend that shoreside measurement be maintained. In particular, for collections that contain a large amount of other organisms (e.g. salps) which makes it difficult to count the eggs on aboard the ship, shoreside counting would be necessary.

Stratified design

An important feature of the survey design was the stratification of sampling by egg density and sampler type. In the high-density stratum, we used only staged eggs from CalVET samples to estimate z and P_0, whereas in the low-density stratum we collected only CUFES samples. We believe it would not be useful to use staged eggs from CUFES samples in the low-density stratum to estimate z and P_0 directly because of the low egg abundance, possibility of stage-specific bias, and lack of yolksac larvae (the CUFES does not sample yolksac larvae well). It seemed preferable to use, for the low-density stratum, the estimate of P_0 for the high-density stratum, adjusted by the ratio of egg densities taken in CUFES at high and low strata. This, of course, requires the assumption that egg mortality did not differ between strata. A direct test of this assumption is impractical because of the large sampling effort needed at low density to obtain a sufficient number of positive samples to detect a difference in mortality rates. Fortunately, the effect of this potential bias is diminished because the low-density stratum contributes fewer eggs. In our example, the low-density stratum contributed about 25%[5] of the daily production. An alternative approach would be to allocate CalVET sampling to the low-density stratum. We believe this approach would not be cost effective because the number of positive CalVETs would be so low.

Another important element of the stratified design was the use of transect lines as the sampling unit. Model-based geostatistics are needed (Fletcher and Sumner, 1999) for data from continuous samplers such as echo-sounders and CUFESs, unless sampling units are defined such that data are uncorrelated among sampling units in the survey design (Armstrong et al., 1988). Because conventional design-based statistical procedures are easier to apply, we preferred using a transect line as our sampling unit, which requires that the minimum sample size allows a between-transect distance greater than the diameter of the egg patch. Fortunately the within-transect CUFES collections provided the information needed on the spatial structure to determine the distance of CalVET lines to insure samples are uncorrelated. In our case, tows, a minimum of 22.2 km

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[4] Castillo Valderrama, P. R. 1995. Distribucion de los principales recursos pelagicos durante los veranlos de 1992 a 1994. Instituo Del Mar Del Peru, Informe No 114. Instituto del Mar delPeru (IMARPE), Esquina Gamarra Y General Valle Chucuito, Callao Peru, 24 p.

[5] 25% =100 × (1.064×107,255)/(2.57×174,096) (Table 4).

(12 nmi) apart, would ensure uncorrelated samples and provide the unbiased estimates of abundance of eggs in each development stage of sardine eggs.

Use of yolksac larvae

Although yolksac larvae have been used to estimate P_0 with the DEPM method (Lo, 1985; 1986; Lo et al., 1996; Hunter and Lo, 1997), but are not a requirement for using the CUFES, we included yolksac larvae to estimate P_0 because the mortality rate of eggs and yolksac larvae are similar for northern anchovy (Lo, 1985, 1986) and because the development of early stages of anchovy and sardine is similar (Ahlstrom, 1943; Zweifel and Lasker, 1976; Moser and Alhstrom, 1985; Lo et al., 1996). Our threshold for taking CalVET samples, 2 eggs per minute, generated 102 positive CalVETs (72% were positive). This number is far fewer positive tows than the number needed to be assured a significant slope for the regression of numbers of eggs on their ages. In anchovy, where the eggs are less patchy than sardine, about 500 positive tows were required to assure a CV of the estimate of mortality rate close to 0.6 (Lo, 1997). To obtain a significant slope in the 1997 survey, we used the number and average age of yolksac larvae (adjusted for observed mean temperature) as well as staged eggs. This introduced a potential bias, because by doing this we assumed that yolksac larvae have the same mortality rate as eggs. To avoid this bias, the number of staged egg samples should be increased. One approach would be to stage the eggs taken with a CUFES in the high-density stratum and combine them with the CalVET samples from the same stratum. This would greatly increase the number of staged eggs available but requires the assumption that CUFES staged eggs are an unbiased sample of the full-water-column, a condition not met in our 1996 pilot study. Another approach would be to increase the number of CalVET samples per mile in the high-density stratum from the present one sample per 4 nmi to a higher frequency. A doubling of the CalVET sampling rate would significantly increase survey costs and may not increase the number of positive samples sufficiently to be able to use only eggs for estimation of P_0. Considering the relative risks and costs of these approaches, we feel that the use of the yolksac larvae in the estimation of P_0 was preferable. In other species, the distribution of eggs may be less concentrated than they are for sardine and sampling at the 4 nmi sampling rate may be adequate. Clearly, other solutions may exist, and we recommend considering these issues with each new application.

Conclusions

We conclude that the CUFES is a useful tool with the DEPM when it is used adaptively to establish sampling strata for CalVETs. In this mode, the CUFES increases precision and reduces cost per transect mile. Perhaps one of the major benefits of using a CUFES in the DEPM is that one can better afford to expand survey boundaries and thereby reduce the potential bias of not enclosing the entire population, a very common bias in field surveys in general (Gunderson, 1993). Clearly, the more contiguous the distribution of spawned eggs within the surveyed habitat, the greater the benefits in using the CUFES.

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Growth of juvenile red king crab (*Paralithodes camtschaticus*) in Bristol Bay (Alaska) elucidated from field sampling and analysis of trawl-survey data

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Abstract—An analysis of *in situ* growth rate was conducted for juvenile red king crab (*Paralithodes camtschaticus*) in Bristol Bay, Alaska. Growth of early juveniles (~2–40 mm CL; age 0–3 yr) was determined by fitting seasonalized Gompertz growth models to length-frequency data. The parameters of the growth model and resulting size-at-age estimates were compared with those from studies conducted at Unalaska and Kodiak Islands by fitting the same growth model to published length-frequency data from separate sources. Growth of late juvenile and early reproductive crabs, ~30–100 mm carapace length (CL), was examined by analyzing length-frequency data from the National Marine Fisheries Service annual Bering Sea trawl survey from 1975 through 1999. Mean CL associated with strong size modes of crabs in Bristol Bay length-frequency distributions was resolved by using the FiSAT software package (FAO-ICLARM Stock Assessment Tools) to track the modal size progression of strong year classes and assign mean size-at-age characteristics to the stock.

Growth of early juvenile crabs was slower in Bristol Bay than that observed by other researchers at Unalaska or Kodiak. Sizes at 1, 2, and 3 years after settlement were estimated to be ~9 mm, 23 mm, and 47 mm CL in Bristol Bay compared with 16 mm, 38 mm, and 66 mm CL at Unalaska; at Kodiak, estimated sizes of 12 mm and 42 mm were obtained for age-1 and age-2 crabs, respectively. Within the Bristol Bay trawl survey data, a total of 24 modes were identified for both males and females <~100 mm CL, which included the modal progression of two year classes that presumably settled in 1976 and 1990. The 1976 year class grew slowly and would not have recruited to the reproductive stock until ~9 years after settlement, whereas the 1990 year class appeared to recruit at ~8 years after settlement. Both estimates indicate that Bristol Bay red king crabs are older at reproductive maturity than the ~6 years after settlement presently assumed. An attempt to resolve discrete mean size-at-age from the length-frequency data met with little success because variability in growth between year classes markedly obscured size-at-age characteristics in the stock.

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Population abundance of red king crab (*Paralithodes camtschaticus*) in Bristol Bay, Alaska (Fig. 1) is typified by great variability. The maximum abundance of harvestable male crabs in the stock over the last 25 years has fluctuated by over an order of magnitude, peaking at nearly 60 million individuals in 1977, and has fallen to less than 3 million from 1983 to 1985 (Loher et al., 1998; Zheng and Kruse[1]). Strong fisheries in the late 1970s and early 1980s were followed by substantially reduced abundance in recent years, and fishery closures in 1981 and 1994, leading to concern over the status of the population by both management agencies and fishermen. Such concern prompted the Alaska Department of Fish and Game to develop a detailed harvest-based recovery plan for the fishery (Zheng et al.[2]) that considers the stock to be fully recovered once it reaches an effective spawning biomass of 55 million pounds. This biomass has not occurred in Bristol Bay since 1981 (Zheng and Kruse[1]; Zheng et al.[2]), but it was chosen as an appropriate rebuilding level primarily on the basis of length-based recruitment models that, in turn, rely on the inferred underlying stock-recruitment relationship for the population (Zheng et al., 1995a, 1995b).

Present assumptions regarding red king crab growth rates suggest that Bristol Bay crabs recruit to the reproductive stock at an age of approximately 6 years after settlement (i.e. ~seven years following egg fertilization; Zheng et al., 1995a, 1995b), which corresponds to mean sizes of 105 mm carapace length (CL) and 97 mm CL for males and females, respectively. These size-at-age values are based on Stevens and Munk (1990), Weber (1967), and Balsiger (1974). However, none of these sources represents a comprehensive treatment of growth from settlement through maturity based entirely on field-collected data from the Bristol Bay region. Balsiger's (1974) growth model was derived from data from Bristol Bay, but it considered only individuals >81 mm carapace length, well above the size at which multiple yearly molts are expected. Weber's (1967) work was based on juvenile crabs collected more than 40 years ago at Unalaska Island (Fig. 1) in the eastern Aleutian Islands, which is located west of Unimak Pass and the coastal shelf break and which is oceanographically separated from Bristol Bay by the southern origins of the Bering Slope current. Stevens and Munk's (1990) growth model was based on data from the Kodiak region (Fig. 1); a separate growth model was developed by Stevens (1990) to consider the eastern Bering Sea, but it relies on Weber's (1967) findings regarding the growth of early juvenile crabs.

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[2] Zheng, J., M. C. Murphy, and G. H. Kruse. 996. Overview of population estimation methods and recommended harvest strategy for red king crabs in Bristol Bay. Alaska Department of Fish and Game, Reg. Inf. Rep. 5J96-04. Division of Commercial Fisheries, Alaska Department of Fish and Game, P.O. Box 25526, Juneau, Alaska, 99801.

Accurate growth rate information is important to properly calibrate the length-based recruitment model and also to determine appropriate time lags between spawning and subsequent recruitment. Given the likelihood of temporal and geographic variability in growth rates, it is likely that the Bristol Bay stock exhibits growth rates different from those observed at different locations by the aforementioned researchers. Environmentally induced changes in molt schedule resulting in considerable variability in size-at-age is a common feature in Crustacea (e.g. Hartnoll, 1982; Hill et al., 1989; Huner and Romaire, 1990; Wainright and Armstrong, 1993; Tremblay and Eagles, 1997) and probably also in red king crab (Stevens, 1990; Stevens and Munk, 1990). Thus, further analysis of the growth of prerecruit crabs within Bristol Bay is needed. In our study, we analyzed growth of early juvenile red king crabs in Bristol Bay by fitting growth equations to length-frequency data collected between 1983 and 1991. We compared the inferred growth rate for Bristol Bay with that determined from identical models fitted to Weber's (1967) data, and with two sets of length-frequency data available for the Kodiak region (Dew, 1990; Donaldson et al., 1992) to assess whether application of these growth rates to Bristol Bay crabs is appropriate. We then expanded our analyses to include older prerecruit crabs in Bristol Bay by analyzing length-frequency distributions from 25 years of southeast Bering Sea trawl survey data in order to identify growth patterns associated with strong year classes and in order to elucidate size-at-age characteristics displayed by the population.

Materials and methods

Growth of early juvenile red king crab

Growth of early juvenile crabs, from settlement through approximately 3 years after settlement, was examined by using catch data from targeted sampling in Bristol Bay obtained from three sources: 1) work conducted in 1983 under the auspices of the Outer Continental Shelf Assessment Program (OCSEAP) (documented in McMurray et al.[3]), 2) OCSEAP work conducted in 1985 (previously unpublished), and, 3) work conducted in 1991 by the National Marine Fisheries Service (NMFS; documented in Stevens and MacIntosh[4]). In addition we reviewed the results of Weber (1967), who collected early juvenile red king crabs at Unalaska Island in 1958 and 1959, as well as data collected in the Kodiak region from 1987 to 1989, originally published in Dew (1990), and from 1990 to 1991, originally published in Donaldson et al. (1992). Note that, in the present paper, all ages and year-class designations are referenced to approximate settlement date. That is, they refer to the postsettlement age of benthic crabs and do not include the larval phase or the egg incubation period. This postsettlement age should be taken into consideration for applications in which the year when eggs were extruded is important.

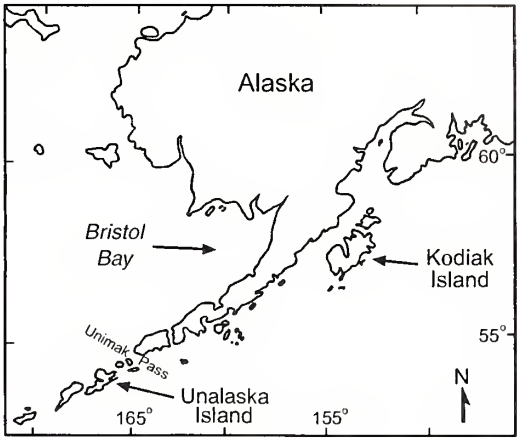

Figure 1

Map of western Alaska, showing the locations of Bristol Bay, Unalaska Island, and Kodiak Island.

Bristol Bay Waters of the Bering Sea east of 163.5°W longitude and south of 59°N latitude will be considered "Bristol Bay." This area is larger than the area that is often referred to as Bristol Bay; it is more typically considered the "southeast Bering Sea." However, because we also report information from Unalaska Island, also in the southeastern Bering Sea, we choose to make a distinction between Bristol Bay and Unalaska Island in order to avoid confusion. In 1983, surveys of juvenile red king crab abundance were conducted throughout the Bristol Bay region (Fig. 2A) with surface-deployed try-net otter trawls and a rock dredge, during three sampling periods: 18 April–7 May, 2–17 June, and 9–23 September (McMurray et al.[3]).

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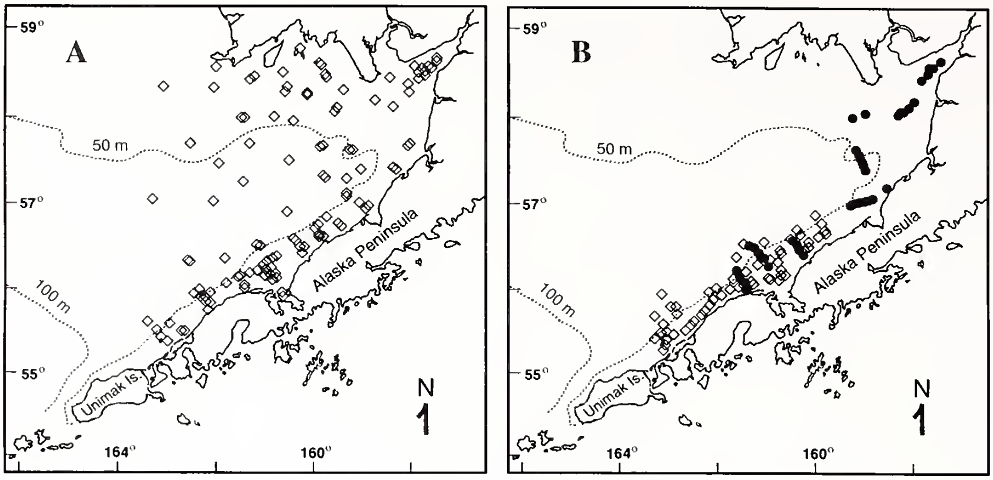

Figure 2

Location of survey stations in Bristol Bay (east of 163.5° west longitude) visited during 1983 OCSEAP studies (**A**), 1985 OCSEAP studies (**B**, open squares), and by Stevens and MacIntosh in 1991 (B, closed circles).

Try nets had a headrope length of 5.4 m and either wooden or aluminum doors measuring 0.4 m wide × 0.9 m tall. The rock dredge was constructed of a rigid steel frame with a mouth opening 0.9 m wide × 0.4 m tall. The 1985 survey was conducted from 19 July to 2 August, nearshore (generally inside the 50-m isobath), along the north Aleutian Shelf from Unimak Pass through the Port Moller region (Fig. 2B) also with try net and rock dredge. The 1991 survey (Stevens and MacIntosh[4]) was conducted from 26 May to 1 June along the North Aleutian Shelf from Port Moller to Kvichak Bay (Fig. 2B) with a 3.1-m wide beam trawl. The carapace length (CL) of all red king crab captured in the above surveys was measured and recorded onboard the vessels.

For each sampling period, length-frequency histograms were constructed to identify individual age classes (cohorts) within the data. Male and female crabs were pooled because sex-specific growth rates are not apparent until reproductive age (Weber, 1967; Dew, 1990), and the combined length-frequency data were analyzed by using the FiSAT software package (Gayanilo and Pauly, 1997) to determine the mean size (±1 SD) for each identifiable cohort. FiSAT employs a combination of Bhattacharya's method (Bhattacharya, 1967) and NORMSEP (Hasselblad, 1966; Pauly and Caddy[5]) to decompose complex size-frequency distributions into a series of best-fit normal curves that represent each cohort within the data set. In our study, the "mean size" of a cohort refers to the mean (±1 SD) of its associated best-fit normal curve, as determined by FiSAT size-frequency decomposition.

Postsettlement age, in Julian days, was then calculated for each cohort. For each year's length-frequency histogram, the cohort with the smallest mean CL was assigned age 0, and the subsequent sizes assigned age 1 and age 2 (Fig. 3). The age of each cohort (in days) was calculated as the time from settlement in the cohort's settlement year until the median sampling date for the survey period. Settlement was estimated to be 15 July of each year because numerous 2-mm-CL individuals occurred in late July during the 1983 surveys, and a carapace length of 2 mm is typical of the first benthic instar (Kurata, 1961; Donaldson et al., 1992; Loher and Armstrong, 2000). Postsettlement age was plotted against the associated mean CL and two growth curves were fitted to the data. The first curve was a seasonalized version of the von Bertalanffy growth model obtained from Anastacio and Marques (1995), where growth in carapace length is expressed as

$$L_t = L_{max}\left\{1 - e^{-[K\times D\times(t-t_{min})+C\times(K\times D/2\Pi)\times\sin 2\Pi t\times(t-t_s)]}\right\}^{(1/D)}, \quad (1)$$

The second curve was a seasonalized Gompertz model, where growth in carapace length is expressed as

$$L_t = L_{max}\left\{e^{-e^{-[K\times(t-t_{min})+C\times(K/2\Pi)\times\sin 2\Pi t\times(t-t_s)]}}\right\}, \quad (2)$$

where (in both models) L_t = carapace length at time t;
L_{max} = maximum carapace length;
t = given time;
t_{min} = time at which the carapace length is the minimum size for the life-stage of interest (in this case, minimum size for benthic red king crab=2 mm);

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[5] Pauly, D., and J. F. Caddy. 1985. A modification of Bhattacharya's method for the analysis of mixtures of normal distributions. FAO Fisheries Circular 781, Sales and Marketing Group, Information Division, FAO, Viale delle Terme di Caracalla, 00100 Rome.

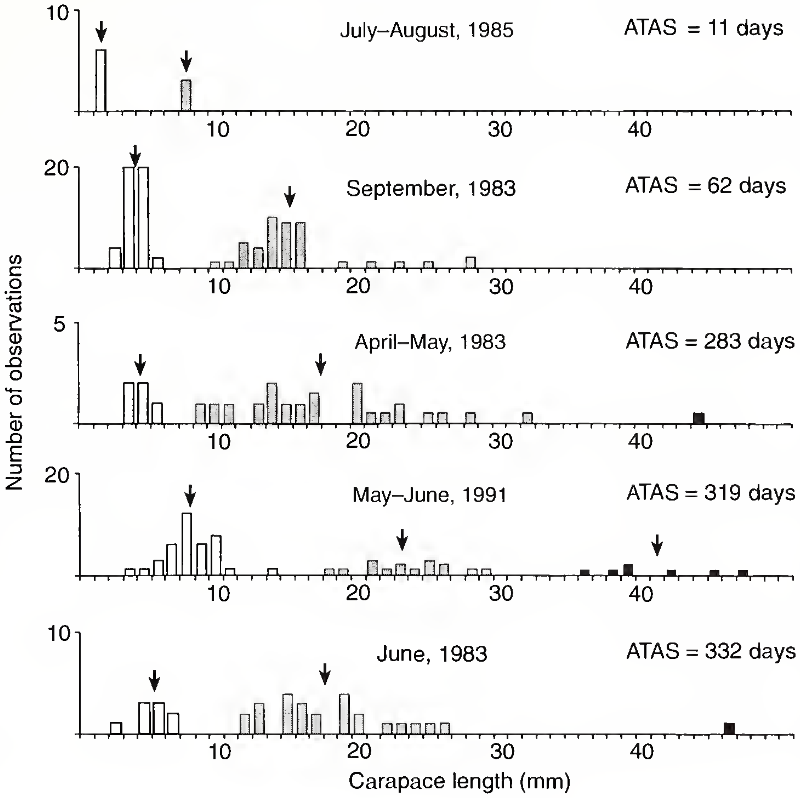

Figure 3

Length-frequency histograms of early juvenile red king crabs captured in Bristol Bay from 1983 to 1991. Each age class is denoted by shading: open bars = age 0+; shaded bars = age 1+; closed bars = age 2+. For each sampling date an average time after settlement (ATAS) is reported. The ATAS is the number of days estimated to have elapsed between 15 July of the sampling year (the most recent settlement event) and the median sampling date. For example, the ATAS for crabs sampled on 15 September was 62 days. This value corresponds to the estimated age of age 0+ crabs in that sample, whereas age 1+ crab would have been ~365 days older than the ATAS, and age 2+ crabs ~730 days older than the ATAS. Note that the June samples fell at nearly one calendar year following the previous year's settlement; hence, age 0+ crab in those samples were nearly age 1. Arrows indicate the mean CL of each size cohort, as determined with FiSAT; mean values were used to fit the growth curve.

t_s = lag-time between the start of growth and the first seasonal growth oscillation; oscillations are sinusoidal with a one-year period;

K = intrinsic growth rate;

C = parameter ranging from 0 to 1 that controls the strength of the seasonal growth oscillation; 0 = no seasonal signature; 1 = strong seasonality with a brief period each year during which growth ceases; and

D = parameter expressing metabolic deviation from the von Bertalanffy 2/3 metabolic rule (in our study, D = 1 [no deviation]).

Unalaska and Kodiak islands Growth rate of early juvenile red king crab at Unalaska Island was assessed by using data published in Weber (1967). In that study immature crabs were collected, primarily with SCUBA, during four sampling periods in 1958 (22 April–17 May; 30 May–6 June; 13 July; 17 September–1 October) and two periods in 1959 (11–24 February; 24 May–2 June). Two data sources were used that originated in Kodiak Island: 1) Donaldson et al. (1992), who documented growth of red king crab in artificial habitat collectors for approximately one year after settlement between June 1990 and May 1991, and; 2) Dew (1990) who collected data on podding age 1+ to 2+ red king crab using SCUBA observations between 20 November 1987 and 3 June 1989. The data in Weber (1967) and Dew (1990) were used to construct length-frequency histograms (males and females pooled) and the characteris-

Table 1

Points used to construct the growth curve for early juvenile red king crab in Bristol Bay. Data originally presented in Stevens and MacIntosh[4] are indicated as such; previously unpublished data are listed as "new."

Collection date	Source	Estimated age (days after settlement)	*n*	Size range (mm)	Mean size ±1 SD
19 Jul–2 Aug 1985	new	11.5	6	2	2.00 ±0.000
10 Sep–19 Sep 1983	new	62	134	3.0–6.0	3.97 ±0.581
21 Apr–6 Jun 1983	new	283.5	10	4.0–6.0	4.27 ±0.730
26 May–1 Jun 1991	Stevens and MacIntosh	319.5	35	4–14	7.70 ±1.645
5 Jun–16 Jun 1983	new	332	13	3.0–7.2	5.19 ±0.982
18 Jul–2 Aug 1985	new	376.5	3	8.0–8.4	8.13 ±0.231
10 Sep–19 Sep 1983	new	427	45	10–28	14.63 ±3.447
21 Apr–6 May 1983	new	648.5	30	10–32	16.70 ±5.389
26 May–1 Jun 1991	Stevens and MacIntosh	684.5	16	18–29	22.53 ±3.562
5 Jun–16 Jun 1983	new	697	25	12–26	17.05 ±4.102
26 May–1 Jun 1991	Stevens and MacIntosh	1049.5	7	34–47	40.36 ±3.681

tics of individual cohorts were resolved by using FiSAT. Donaldson et al. (1992) did not provide their original data; therefore we simply used the mean size-at-age values that they reported. Mean size-at-age values for each region were then fitted with seasonalized von Bertalanffy and Gompertz curves, as described previously. Age of crabs at Unalaska Island was calculated by using 1 July as the approximate settlement date, as suggested by Weber (1967); 14 June was used for Kodiak Island because Donaldson et al. (1992) first collected benthic instars on this date.

Mean size-at-age of late juvenile through early reproductive-age crabs

Growth of late juvenile (~age 2+) through early reproductive-age crabs was examined by using data from the annual NMFS groundfish trawl survey from 1975 to 1999 (please refer to Otto [1986] for a description of the annual trawl survey protocol and its spatial coverage). These data were used to construct annual length-frequency histograms for the Bristol Bay stock; length-frequency distributions were examined to identify strong size modes, and the mean CL (±1 SD) associated with each mode was determined by using FiSAT. Two approaches were then employed to assign discrete mean size-at-age categories to the population, focusing on size modes with mean CL <100 mm. First, each strong year class that recruited to the population was identified, and the growth of individuals in these year classes was determined by examination of progression of their length-frequency modes, from the first appearance of the year classes until they could no longer be resolved. Second, the mean CL associated with every strong size mode that appeared in all the annual length-frequency distributions, over the entire time series, was plotted to identify commonly occurring mean CLs that might represent consecutive size-at-age categories.

Bottom temperature

Area-averaged near-bottom temperature was calculated for each year from 1975 to 1999, to more fully interpret the growth rates observed in Bristol Bay region with respect to variation over time. The area-averaged near-bottom temperature represented the mean temperature over all trawl-survey stations located within the Bristol Bay region. Because the survey data were sometimes incomplete and sampling stations were not located in precisely the same location each year, the temperature at the center of each trawl survey station was statistically interpolated with known values at surrounding points (i.e. "kriged"; see Cressie, 1993). Kriging was performed with the Surfer 6.04 software package (Keckler, 1994) and a linear variogram model (Cressie, 1993). Area-averaged near-bottom temperatures for the Bristol Bay region were then calculated by using the kriged estimates obtained from the center of trawl survey stations.

Results

Early juvenile red king crab

Resolution of age classes from length-frequency data collected in Bristol Bay in 1983, 1985, and 1991 provided 11 estimates of mean size-at-age representing individuals ranging from approximately 12 to 1050 days after settlement (refer to Fig. 3, Table 1). Weber (1967) provided information resulting in 24 mean size-at-age estimates (Table 2) at Unalaska, for crabs estimated to be ~86–964 days after settlement. Donaldson et al. (1992) and Dew (1990) provided 22 estimates of mean size-at-age for crabs ~0–720 days after settlement (Table 3) at Kodiak Island. Plots of early juvenile mean size-at-age are presented in Figure 4 for Bristol Bay, Unalaska Island, and

Table 2

Points used to construct the Unalaska early juvenile red king crab growth curve from data of Weber (1967) (his Appendix Table II). The original data come from crabs that were collected at a number of study sites around Unalaska Island; the "sample area" column in this table refers to different study sites as designated by Weber (1967). Refer to the original paper for descriptions of each specific sample area.

Collection date	Sample area	Estimated age (days after settlement)	Molt status	Size range (mm)	Mean size (mm) ±1 SD
17 Sep–1 Oct 1958	2	86	not reported	4–6	4.81 ±0.634
11–24 Feb 1959	2	232.5	postmolt	7–10	8.32 ±0.750
22 Apr–17 May 1958	2	309	not reported	8–17	12.62 ±1.799
24 May–2 Jun 1959	2	333.5	pre- and postmolt	8–16	11.26 ±1.406
30 May–6 Jun 1958	2	338.5	not reported	9–18	12.73 ±1.433
13 Jul 1958	2	379	not reported	13–18	16.06 ±0.869
17 Sep–1 Oct 1958	2	452	not reported	15–34	24.08 ±3.994
17 Sep–1 Oct 1958	4	452	not reported	17–29	20.22 ±1.500
11 –24 Feb 1959	2	598.5	premolt	23–35	29.62 ±2.376
11–24 Feb 1959	2	598.5	postmolt	25–44	35.12 ±3.798
11–24 Feb 1959	4	598.5	pre- and postmolt	22–40	30.56 ±4.200
22 Apr–17 May 1958	1	674	not reported	29–35	31.85 ±1.682
22 Apr–17 May 1958	2	674	not reported	23–48	36.68 ±4.392
24 May–2 Jun 1959	4	698.5	premolt	28–41	29.10 ±2.783
24 May–2 Jun 1959	4	698.5	postmolt	31–41	33.73 ±2.919
24 May–2 Jun 1959	2	698.5	premolt	25–41	35.00 ±2.388
24 May–2 Jun 1959	2	698.5	postmolt	28–51	39.65 ±4.238
30 May–6 Jun 1958	2	703.5	not reported	29–50	39.75 ±4.373
17 Sep–1 Oct 1958	4	817	not reported	41–53	45.86 ±3.308
11–24 Feb 1959	1	963.5	premolt	33–51	40.85 ±3.162
11–24 Feb 1959	1	963.5	postmolt	41–69	49.17 ±4.925
22–24 Feb 1959	3	963.5	premolt	42–74	52.70 ±6.000
11–24 Feb 1959	3	963.5	postmolt	51–82	66.23 ±8.030
11–24 Feb 1959	4	963.5	premolt	43–78	57.19 ±7.659

Kodiak Island data. Seasonalized Gompertz growth functions were fitted to the data; von Bertalanffy growth curves are not presented because their fits were poorer by comparison (Table 4). Model fits and parameter estimates for Gompertz growth functions are reported in Table 5, along with model estimates of CL at ages 1.0 and 2.0 at all sites, and at age 3.0 for Bristol Bay and Unalaska Island; predicted sizes at ages 0.9, 1.9, and 2.9 are also reported because the Bering Sea trawl survey occurs ~0.1 years prior to the settlement season. No data were available for Kodiak Island crabs beyond age ~2; therefore model predictions are not reported beyond this age.

Intrinsic growth rate was lowest in Bristol Bay and was coupled with a very strong seasonal signature, with no growth expected during mid-winter. The growth rate observed at Kodiak Island was higher than in Bristol Bay, with a moderate seasonal signature, whereas

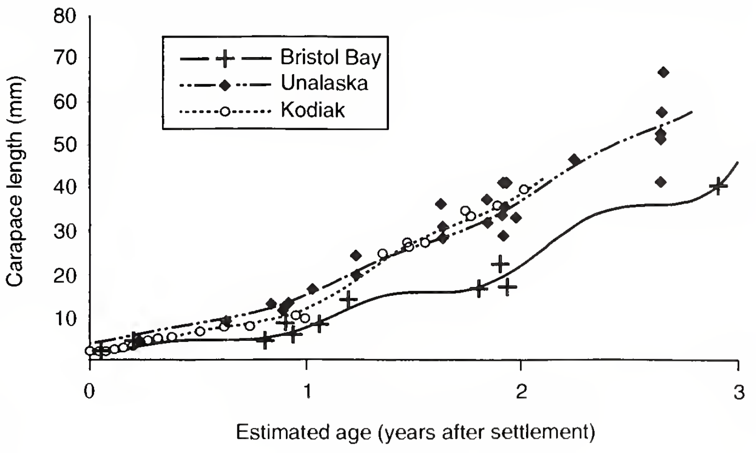

Figure 4

Seasonalized Gompertz growth curves fitted to mean size-at-age data from Bristol Bay, Unalaska Island, and Kodiak Island. Note that no data were available for Kodiak crabs > ~2 years after settlement.

Table 3

Points used to construct the Kodiak early juvenile red king crab growth curve. Data from Donaldson et al. (1992) come directly from their Table 1; data from Dew (1990) are compiled from his Figures 7 and 8.

Collection date	Estimated age (days after settlement)	Size range (mm)	Mean size (mm) ±SD
Donaldson et al. (1992)			
14 Jun 1990	0	1.9–2.4	2.18 ±0.118
28 Jun 1990	14	1.8–2.6	2.18 ±0.171
13 Jul 1990	29	2.0–3.3	2.61 ±0.363
26 Jul 1990	42	2.5–3.4	2.84 ±0.152
10 Aug 1990	57	2.4–4.2	3.64 ±0.408
23 Aug 1990	70	2.6–5.4	3.78 ±0.362
7 Sep 1990	85	3.4–5.1	4.47 ±0.421
21 Sep 1990	109	4.2–6.4	4.98 ±0.605
16 Oct 1990	124	3.4–6.1	5.29 ±0.491
4 Dec 1990	173	4.6–7.9	6.58 ±0.703
11 Feb 1991	212	4.6–9.0	7.71 ±1.201
27 Mar 1991	257	6.1–10.3	7.95 ±0.769
14 May 1991	335	8.7–13.0	10.46 ±1.079
29 May 1991	350	8.6–9.9	9.33 ±0.474
Dew (1990)			
6 Oct 1988	480	17.5–34.0	25.67 ±2.446
18 Nov 1988	523	19.0–35.5	27.87 ±2.795
20 Nov 1987	525	22.0–32.5	26.93 ±2.572
18 Dec 1987	553	22.0–27.4	28.10 ±2.726
24 Feb 1989	621	25.0–43.0	35.27 ±3.693
8 Mar 1988	641	22.0–41.5	34.09 ±3.688
19 Apr 1988	675	26.5–44.5	36.49 ±3.176
3 Jun 1988	720	31.0–49.0	40.18 ±3.361

growth rate observed at Unalaska Island was most similar to that at Kodiak Island, and very little seasonal signature could be detected. Estimated mean size at age 2 at Unalaska Island (37.6 mm CL) was much closer to the value estimated for Kodiak Island (42.2 mm CL) than for Bristol Bay (22.7 mm CL, Table 5); at age 3, Bristol Bay crabs were expected to average ~20 mm smaller than crabs observed at Unalaska Island. It is important to note that the choice of settlement dates for the models had negligible effect on size-at-age estimates, except where such changes caused cohorts to be re-assigned to younger age classes than seemed reasonable (i.e. if early spring settlement dates had been chosen, very small crabs would have been assigned to late age 0, instead of being considered immediately postsettlement crabs). Changes in settlement date primarily affected estimated size-at-age over the first few months but had little impact on estimation of size at 1, 2, or 3 years after settlement.

Table 4

Comparison of coefficient of determination (r^2) goodness-of-fit values associated with seasonalized Gompertz and von Bertalanffy growth curves fitted to early juvenile red king crab size-at-age data.

Region	Gompertz	von Bertalanffy
Bristol Bay	0.968	0.842
Unalaska Island	0.922	0.863
Kodiak Island	0.995	0.919

Late juvenile through early reproductive-age crabs

Male red king crabs collected in NMFS trawl surveys from 1975 to 1999 ranged from 6 to 201 mm CL, and all sizes between 23 and 198 mm CL were observed. Female red king crabs ranged from 7 to 192 mm CL; all sizes between 24 and 165 mm CL. Over the entire time series, two particularly strong year classes recruited to the Bristol Bay population whose growth and size-at-age characteristics could be tracked by modal size progression. The first year class was evident from 1979 to 1984, first appearing as size modes with mean CL = ~32 mm and

~30 mm for males and females, respectively (Fig. 5, Table 6); these crabs were most likely ~2.9 years of age and represented the year class that settled in 1976. Males of this year class grew to a mean CL = ~88 mm by 1983 and the mode became indistinguishable in 1984; females grew to a mean CL = ~84 mm by 1983, and ~92 mm by 1984 (Fig. 5, Table 6). The second strong year class was evident from 1994 to 1999 (Fig. 6, Table 6) and first appeared at larger sizes than did the 1976 year class. Males first appeared at a mean CL = ~54 mm and grew to a mean CL = ~137 mm in 1999; females first appeared at ~53 mm CL in 1994 and grew to ~109 mm in 1999. This year class first appeared at sizes that were essentially equivalent to the sizes that individuals from the 1976 year class had attained at age 3.9. Thus, this second year class most likely represented crabs that settled in 1990.

For both year classes, growth of males and females was similar up to ~85 mm CL, after which females grew more slowly than males. However, the growth rates of the two year classes were not equivalent: the 1976 year class grew slower than the 1990 year class (Fig. 7) because the 1976 year class required two years (from 1980 to 1982) to progress from a mean CL = ~50 mm to mean CL = ~70 mm, whereas the 1990 year class achieved this level of growth within a single year (from 1994 to 1995). Mean sizes during the following two years of growth (from 1982 to 1984 for the 1976 year class; 1995 to 1997 for the 1990 year class, Table 6) were similar between year classes.

Within all of the annual length-frequency distributions, considering only size modes with mean CL <100 mm, 24 modes were identified for both males and for females. These included the modes presented previously for the 1976 and 1990 year classes, and an additional 16 modes for males, and 14 modes for females. The additional modes represent other year classes whose modal progression could not be tracked for a substantial period of time. The mean CL (±1 SD) of all 24 male and female modes is presented in Figure 8, plotted sequentially by increas-

Table 5

Characteristics of seasonalized Gompertz growth curves fitted to size-at-age data from Bristol Bay, Unalaska Island, and Kodiak Island. Values are reported for ages 0.9, 1.9, and 2.9 because these ages roughly correspond to trawl survey data: the trawl survey typically occurs in late May, ~0.9 years following the previous year's settlement. For all curves, L_{max} = 200 mm CL. See "Materials and methods" section for definitions of model parameters.

Model parameters	Bristol Bay	Unalaska Island	Kodiak Island
r^2 (fit)	0.968	0.922	0.995
K	0.415	0.421	0.634
C	1.000	0.275	0.599
t_s (year)	0.553	0.535	0.680
t_{min} (year)	–3.922	–3.286	–2.659
Length estimates from the models			
age 0.0	3.1	3.6	2.7
age 0.9	7.0	14.5	9.9
age 1.0	8.5	16.4	11.7
age 1.9	19.5	34.5	38.0
age 2.0	22.7	37.6	42.2
age 2.9	41.9	62.7	—
age 3.0	46.7	66.4	—
Slowest growth in	January	January	February

Table 6

Summary of mean size (±1 SD) of the cohorts depicted in size-frequency progression plots (Figs. 5 and 6), as determined with FiSAT.

Year class	Year	Male mean size (mm) ±1 SD	Female mean size (mm) ±1 SD	Age (years after settlement)
1976	1979	31.9 ±2.79	29.6 ±2.04	2.9
	1980	50.7 ±4.10	50.2 ±3.40	3.9
	1981	63.2 ±5.06	64.0 ±5.73	4.9
	1982	70.1 ±7.50	71.4 ±8.20	5.9
	1983	88.4 ±14.74	83.5 ±11.23	6.9
	1984	cohort indistinct	92.3 ±4.84	7.9
1990	1994	54.1 ±4.67	52.7 ±3.75	3.9
	1995	73.4 ±4.90	71.9 ±7.28	4.9
	1996	86.5 ±8.01	83.5 ±5.89	5.9
	1997	104.2 ±8.44	97.4 ±3.62	6.9
	1998	117.8 ±11.40	105.3 ±8.01	7.9
	1999	136.5 ±11.92	109.3 ±7.79	8.9

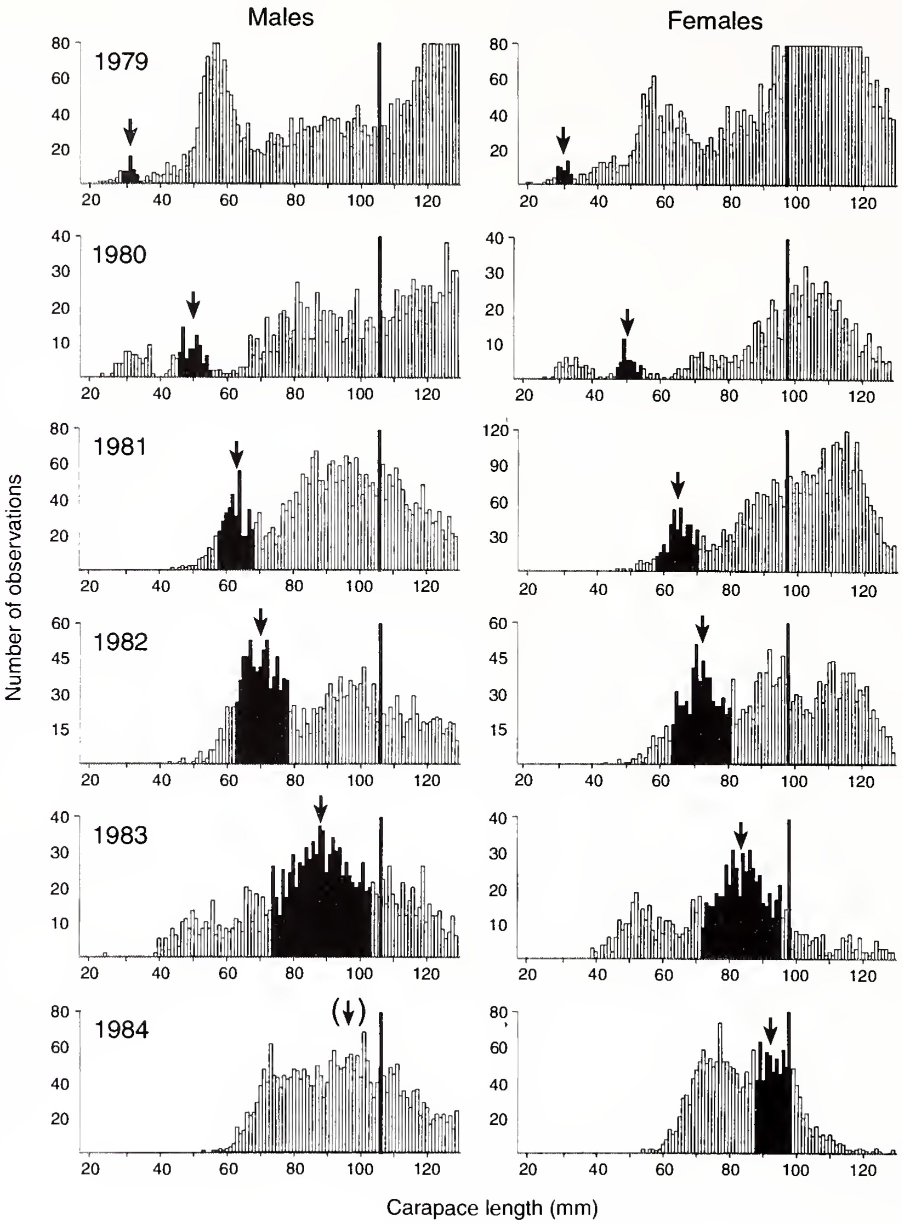

Figure 5

Yearly length-frequency histograms for male (left) and female (right) Bristol Bay region red king crabs <130 mm CL, 1979–1984, revealing mean size-at-age of the 1976 year class by modal progression. Arrows indicate the cohort's mean CL each year, as determined with FiSAT; dark shading indicates CLs within 1 SD of the mean. The vertical lines located at 105 mm CL and 97 mm CL indicate size at reproductive recruitment for males and females, respectively, as defined by Zheng et al. (1995a, 1995b). Note that the 1979 histograms have been truncated at n = 80 observations in order to make the relevant size modes more visible. The 1984 male size mode was not resolved with FiSAT; we considered this size mode to be too indistinguishable from the surrounding data to yield an accurate result.

ing mean CL. This figure represents an attempt to identify commonly occurring sizes that may represent the expected CLs of consecutive age classes. For male crabs, a cluster of three observations occurred at ~35 mm CL, separated from the remaining observations, and likely representing a single age class; for females, two observations occurred at the same approximate size, separated from the remaining modes. Considering the estimated age

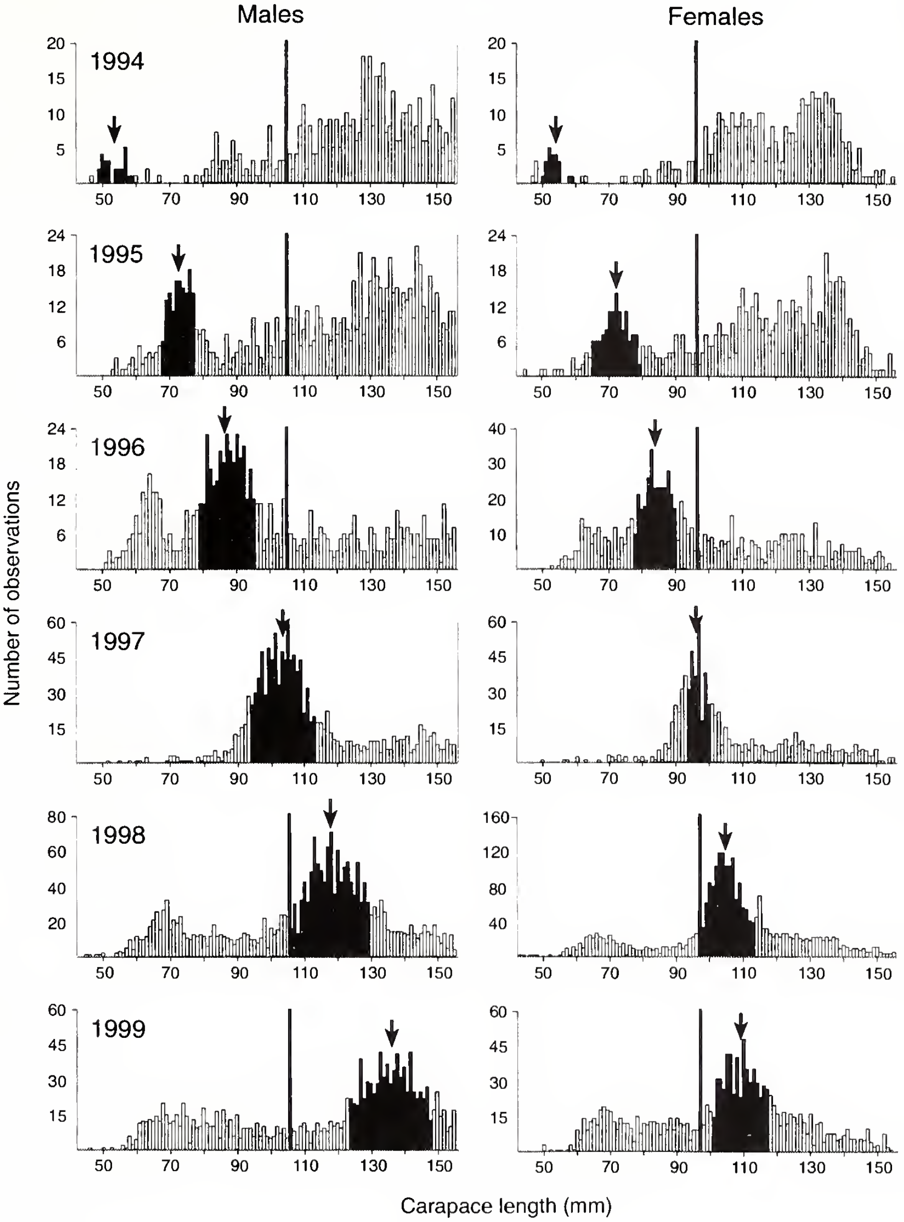

Figure 6

Yearly length-frequency histograms for male (left) and female (right) Bristol Bay region red king crabs <155 mm CL, 1994–1999, revealing mean size-at-age of the 1990 year class with modal progression. Arrows indicate the cohort's mean CL each year, as determined with FiSAT, and dark shading indicates CLs within 1 SD of that mean. The vertical lines located at 105 mm CL and 97 mm CL indicate size-at-reproductive recruitment for males and females, respectively, as defined by Zheng et al. (1995a, 1995b).

at size 2.9 of ~40 mm obtained from the growth curve presented earlier (Fig. 4, Table 5), these small crab are likely age 2.9. At larger sizes, there was considerable overlap between modes, suggesting that the mean CL of older age classes is quite variable and difficult to distinguish among years.

Bottom temperature

From 1975 to 1999, area-averaged near-bottom temperatures in Bristol Bay ranged between a low of 0.7°C in 1976 and a high of 5.2 °C in 1981 (Fig. 9). In general, the coldest temperatures were observed in the 1970s, warmest tem-

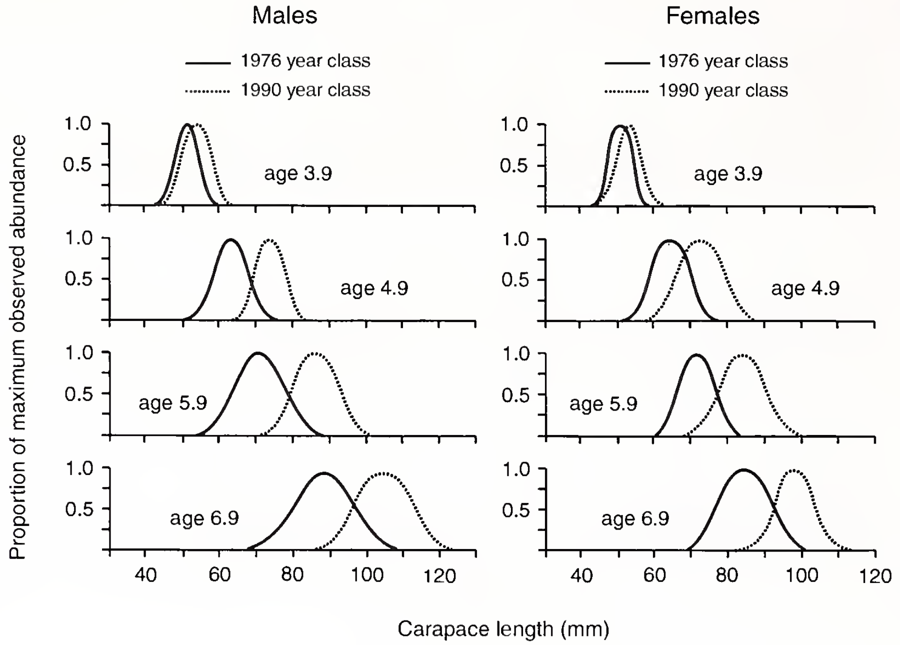

Figure 7

Best-fit normal curves, as determined with FiSAT, of the length-frequency modes associated with male and female red king crabs of the 1976 (solid curves) and 1990 (dashed curves) year classes, from age 3.9 to 6.9, demonstrating the divergence in size-at-age between the two year classes. In particular, note the large difference in apparent molt increment displayed by the year classes between ages 3.9 and 4.9. All curves have been standardized to a uniform height in order to facilitate their comparison, because the number of crabs that were observed differed between years.

peratures during the early 1980s, and moderate temperatures from the mid-1980s through the late 1990s.

Discussion

Our analyses demonstrate that Bristol Bay red king crab grow slower than previously assumed. The present stock-recruitment relationship used to manage this population is based on growth models (Weber, 1967; Balsiger, 1974; Stevens and Munk, 1990) that suggest a time lag of six years between settlement and subsequent recruitment (i.e. seven years after fertilization), where reproductive recruitment is defined to occur at ~97 mm and ~105 mm CL for females and males, respectively (Zheng et al., 1995a, 1995b). Our results indicate that mean age at full reproductive recruitment is likely 8–9 years after settlement. Crabs that settled in 1990 began to reach reproductive size in 1997, at 7 years after settlement, and at this age only ~50% of the individuals were at or above the reproductive size cut-off; the mode did not become fully recruited for an additional 1 to 2 years. Individuals of the 1976 year class grew even slower and, on average, were still slightly smaller than reproductive size at 8 years after settlement. This year class would not have begun to contribute substantially to the reproductive stock until at least 9 years after settlement.

The discrepancy between our estimates of mean age-at-recruitment and presently accepted values is due in part to incorrect assumptions of the latter regarding the growth of early juveniles in Bristol Bay. Weber (1967) conducted one of the few comprehensive *in situ* studies of early juvenile size-at-age in Alaskan waters and clearly demonstrated that red king crab should be expected to reach a mean CL = ~66 mm CL three years after settlement. This conclusion contrasted sharply with earlier data from Bristol Bay that showed strong size modes with means of 4 mm, 9 mm, and 17 mm CL in early summer samples in 1956, 1957, and 1958, respectively (Fisheries Agency of Japan[6]), apparently representing the modal progression of the 1956 year class, and suggesting a much slower growth rate. More recent studies conducted in Kodiak (Dew, 1990; Donaldson et al., 1992) have supported Weber's (1967) conclusions; thus, the observations made by the Fisheries Agency of Japan[6] have been largely ignored. Our results suggest that the data from Kodiak is in good accord with Weber's (1967) conclusions, but that mean CLs of 4 mm,

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[6] Fisheries Agency of Japan. 1959. Report of research on king crab in the eastern Bering Sea. Int. N. Pac. Fish. Comm. Annu. Rep., p. 71–78. [Available from Secretariat, North Pacific Anadromous Fish Commission, Suite 502, 889 West Pender Street, Vancouver, British Columbia, V6C 3BC.]

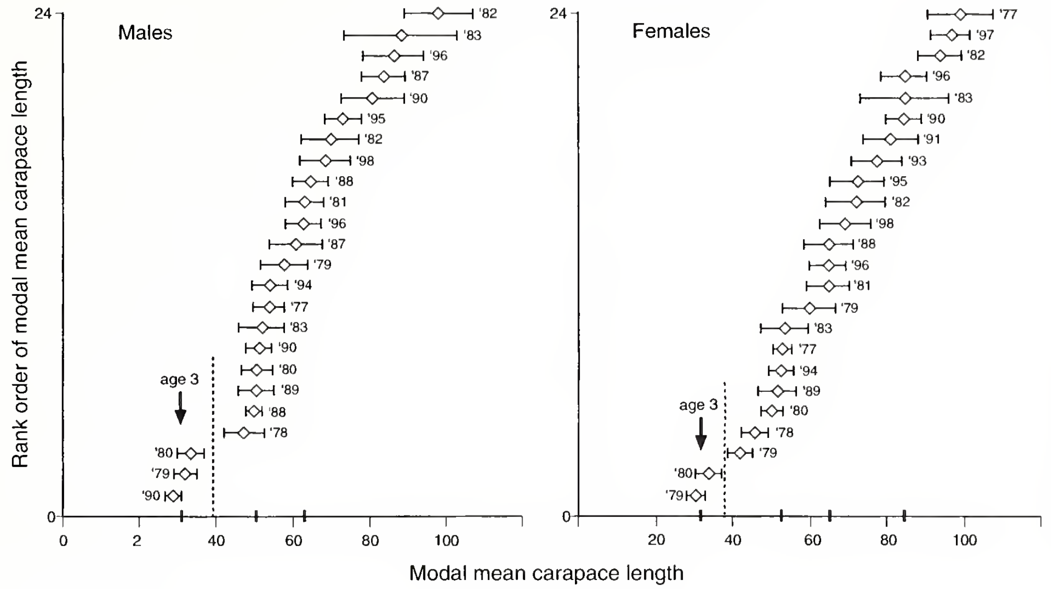

Figure 8

Mean CL ±1 SD of all strong size modes of Bristol Bay red king crabs appearing in the annual length-frequency data from NMFS Bering Sea trawl surveys, 1975–1999; only modes with mean CL <100 mm are plotted. Size modes are plotted in length-sequential order along the *x*-axis, starting with the smallest observed mean CL. The numbers adjacent to each mode indicate the year during which that mode was observed. A grouping of modes separate from the remaining data, indicated by the dashed line and the arrow, likely represents a discrete age class; these small crabs are most likely age 2.9 (refer to Fig. 4). The remaining observations are continuous; substantial size overlap between consecutive modes suggest that discrete size-at-age categories are lacking at >~40 mm mean CL.

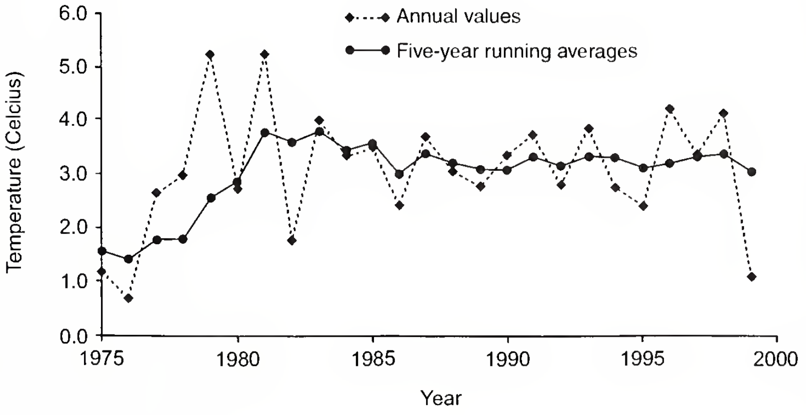

Figure 9

Area-averaged June bottom temperatures in the Bristol Bay region from 1975 to 1999. The five-year running averages represent a five-year period that includes the plotted date and the four years preceding it; for example, the five-year average temperature plotted at 1981 (3.0°C) represents mean area-averaged temperature from 1977 to 1981.

9 mm, and 17 mm are consistent with sizes at age 0, 1, and 2, respectively, within Bristol Bay. This may be due, in part, to reduced molt frequency in Bristol Bay: the data obtained from Weber (1967) indicate that mid-winter molting was not uncommon during his study, whereas our data suggest that this may not be typical in Bristol Bay. It is difficult to determine whether the discrepancies represent actual regional differences, or simply differences between different studies conducted at different sites and at different times, but our results indicate that it is probably inappropriate to apply the early juvenile growth rate obtained by Weber (1967) to the Bristol Bay stock; use of the faster growth rate likely results in lower estimates of age-at-recruitment than the population displays.

Variable growth of older juveniles (age 3+) may further delay age-at-recruitment. This variability is evident when comparing growth of the 1976 year class to that of the 1990 year class; age-at-recruitment was 1–2 years greater in the former, due to slow growth of prerecruits. In particular, note that females of the 1976 year class, averaging 83.5 mm CL in 1983, displayed a mean increase in carapace length during molting (molt increment [MI]) of ~9 mm during the 1984 spring molt, whereas females of the 1990 year class, also averaging 83.5 mm CL in 1996, exhibited a mean MI of ~14 mm during the 1997 spring molt. As a result, females from the 1976 year class required four years to grow from a mean CL = ~50 mm to a mean CL = ~92 mm CL and at ~8 years after settlement were still slightly smaller than the estimated size for full reproductive recruitment (Zheng et al., 1995a, 1995b). Females from the 1990 year class were able to accomplish slightly more mean growth, from ~53 mm to ~97 mm CL, in only three years.

The large difference in growth rate between the 1976 and 1990 year classes may have been caused by water temperature differences during the two time periods. Molt schedules and growth rates can be strongly influenced by ambient temperature (Kurata, 1960, 1961; Nakanishi, 1985), and considerable variability in size at maturity has been observed over the species' geographic range in both males (Paul et al., 1991) and females (see review in Blau, 1990; Otto et al., 1990). Though a number of factors may contribute to the observed variability, reduced growth associated with colder bottom temperatures has been invoked to explain the smaller size-at-maturity observed in the Norton Sound population as compared with other Bering Sea stocks (Blau, 1990; Otto et al., 1990), and modeling suggests that regional and temporal variation in temperature can have broad effects on age-at-recruitment (Stevens, 1990; Stevens and Munk, 1991). June bottom temperature profiles in Bristol Bay suggest that the 1976 year class was subjected to lower temperatures than the 1990 year class, primarily at early juvenile ages. Although a detailed analysis of temperature-dependent growth would require year-round temperature records, which are not available for this region, June temperatures in Bristol Bay may serve as a proxy for thermal conditions throughout the year. Bottom temperatures in Bristol Bay are linked to seasonal sea ice, that in some years covers much of Bristol Bay (NIC, 1994; Wyllie-Echeverria, 1995; Neibauer, 1998), and the development of sea ice can have strong effects on bottom temperature conditions throughout the year. "Cold pool" bottom waters (<1°C) produced in the winter during ice formation may persist well into the summer, and potentially into the following winter, once insulated from surface heating by the development of the summer thermocline (Azumaya and Ohtani, 1995).

In many Crustacea, temperature primarily affects the molt schedule and has little influence on the magnitude of the MI (Hartnoll, 1982; Wainright and Armstrong, 1993), but laboratory studies conducted with red king crab indicate substantial variability in MI-at-age, across ranges of temperatures, as well as under stable environmental conditions. Rearing crabs ~6.3 mm CL under constant temperatures of ~10°C, Molyneaux and Shirley (1988) reported changes in CL at molt that ranged from –4.4% to 52.2%; similarly, for juvenile premolt crabs 33–36 mm CL, reared at ~5.0°C, Gharrett (1986) observed MIs ranging from 3 to 8 mm. At reproductive age, Weber and Miyahara (1962) observed that MI varied between 5 and 23 mm CL per molt in adult males, and large variability in MI associated with water temperatures between 0° and 12°C has been demonstrated for ovigerous females (Shirley et al., 1990).

Because the changes in mean CL the we observed for the Bristol Bay stock were determined through modal analysis of the entire population, it is reasonable to suspect that apparent differences in growth between years and cohorts do not represent MI variability but may be explained as variability in the number of molted versus unmolted crab within particular survey years. This is reasonable to assume, considering that red king crab may skip molting so that the annual molt schedule is replaced by a biennial or triennial cycle (Weber and Miyahara, 1962; McCaughran and Powell, 1977; Balsiger, 1974). However, closer examination of the trawl survey data indicates that, of the 23 size modes of crab observed, none comprised less than 94% new-shelled crabs that had recently molted (senior author, unpubl. data). A high proportion of newly molted crabs was characteristic of nearly all the identifiable size modes (senior author, unpubl. data): of the 53 modes identified, 35 comprised entirely new-shelled crabs, 17 comprised 94–99% new-shelled crabs, and only one comprised >10% old-shell individuals (the female cohort with mean CL=~94mm CL in 1982; population=11.4% old-shell). Thus, the difference in growth rates observed between the two year classes of late juveniles cannot be explained by variations in molt frequency; the trawl survey data support the conclusion that variability in MI is a characteristic shared by both sexes across a range of ages.

Substantial variability in MI is an important life history characteristic that confounds attempts to assign discrete size-at-age categories to a population. The greater the variability in MI among individuals and over time, the greater will be the range of sizes associated with crabs in a given year class, making modes more difficult to resolve from one another. Size-at-age values presently used for Bristol Bay red king crabs were derived from studies consisting of 1–4 years of data (Weber and Miyahara, 1962; Weber, 1967; McCaughran and Powell, 1977; Incze et al., 1986), but our analyses show that a strong tendency toward specific mean size-at-age is not apparent if longer time scales are considered. Size-at-age characteristics may be different depending on which year class is considered, and among crabs >~40 mm CL, mean CLs of identifiable cohorts displayed a fairly continuous distribution with considerable overlap between adjacent size modes; we could not identify specific mean CLs that could be consistently assigned to various age classes. In addition to confounding age estimates, variable MI may cause different year classes to recruit at different ages, over different time spans, and increase the number of year classes that constitute each year's new recruitment. These issues have been treated elsewhere with respect to variable intermolt period (Stevens, 1990); variability in MI will produce the same effects.

We explored the possibility that overlap between size modes might be attributable to changes in growth rate within the population over time. That is, because bottom temperatures in Bristol Bay were colder during the 1970s than they have been more recently, growth was expected to be slower in the 1970s than later in the time series (Stevens, 1990). Thus, one might expect size modes to fall closer together early in the time series and to be spaced farther apart later. However, we were unable to resolve a clear temporal component in the data; even consecutive year classes sometimes had different mean size-at-age and growth increment characteristics. For example, the 1976 year class displayed a mean CL = ~51 mm (range: 44–57 mm) and ~63 mm CL (range: 56–71mm) in 1980 and 1981, respectively. These represented clear and well-separated size modes at ages 3.9 and 4.9, respectively. However, note the occurrence of a strong mode in the 1979 data, with a mean CL = ~58mm CL (range=50–65 mm); this mode probably represents a single year class, settled in 1975, which would be expected to have mean size-at-age characteristics similar to those of the 1976 year class. Yet, this mode of the 1975 year class fell almost precisely between, and its range encompassed the mean sizes of both age 3.9 and age 4.9 crabs from the 1976 year class. Such features were not uncommon in the length frequencies that were presented. For applications requiring accurate size-at-age information, the onerous task of year-by-year and year-class-by-year-class assessments may be necessary.

In summary, our results demonstrate that both male and female red king crab in Bristol Bay reach maturity at least one year later than presently assumed (i.e. at ~7 years after settlement) due to slower growth from settlement through age 3. Furthermore, variability in MI in late juveniles can result in further reduction in growth rate such that reproductive recruitment is delayed by an additional 1–2 years (i.e. reproductive recruitment at ~8–9 years after settlement). From a management perspective, variable MI is a life-history characteristic that should be considered in growth- and length-based models of recruitment. Present models attempt to simulate MI variability (Zheng et al., 1995a), but little information exists on the magnitude of that variability and its changes over time and space. Such information will be valuable to managers to calibrate recruitment models with respect to lag times between spawning and subsequent recruitment, as well as to predict how year classes enter the spawning population and the fishery; more research in this area is warranted. Inappropriate growth rate and lag-time assumptions have resulted in assigning the wrong year's spawning stock biomass to subsequent recruitment levels in the Bristol Bay stock-recruitment curve; spawning stock abundances are presently offset ~1–2 years from the recruitment levels that they generated. This offset may affect the precise shape of the stock-recruitment curve and alter some of the models associated with it. Such changes may prove negligible with respect to actual harvest strategies, but it may be prudent to make the appropriate adjustments given knowledge of greater age at reproductive recruitment.

The Bristol Bay red king crab stock has been typified by large fluctuations in fishable abundance and by relatively rare, strong recruitment pulses generating the bulk of the fishery. Most recently, relatively strong catches from 1997–99, yielding a combined landed catch of ~35 million pounds of crabs with an exvessel value estimated at over $137 million (Morrison et al.[7]), were supported almost entirely by the 1990 year class (i.e. were spawned by the 1989 reproductive stock). Former assumptions would have led us to assign this pulse to the 1990 spawning stock and assume that the planktonic larval phase and settlement occurred during 1991. From a management standpoint, the ramifications of such an error may be negligible because estimated effective spawning biomass was similar in both 1989 and 1990 (Zheng and Kruse[1]). However, as we try to elucidate the mechanisms that generated this strong year class, it is crucial that we accurately determine when those crabs were larvae, early benthic individuals, and later stage crabs. Physical forcing, for example, has been shown to play a large role in determining recruitment variability in a number of commercially important crustacean species worldwide (e.g. Polovina and Mitchum, 1992; Polovina et al., 1993; McConnaughey et al., 1994; Rothlisberg et al., 1994; Jones and Epifanio, 1995; McConnaughey and Armstrong, 1995; Rozenkranz et al., 1998). Similar correlations between recruitment and physical parameters have been attempted for king crabs (Zheng and Kruse, 2000), but our ability to identify causes of recruitment variability relies upon associating the correct life history stages with physical forcing events. Even seemingly minor errors in growth-rate assumptions can have serious impacts on our understanding of population dynamics.

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[7] Morrison, R., F. Bowers, R. Gish, E. Wilson, W. Jones, and B. Palach. 2000. Annual management report for shellfish fisheries of the Bering Sea. *In* Annual management report for shellfish fisheries of the westward region, 1999, p. 147–261. Regional Information Report 4KOO-55, Kodiak. Division of Commercial Fisheries, Alaska Department of Fish and Game, P.O. Box 25526, Juneau, Alaska, 99801.

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Age, growth, and mortality of California halibut, *Paralichthys californicus,* along southern and central California

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Abstract—California halibut, *Paralichthys californicus,* collected by a 400-mesh eastern trawl in southern (Mexican border to Point Conception) and central (Point Conception to Tomales Bay) California were aged by using whole and sectioned otoliths (sagittae) to determine age, growth, and mortality. Males represented 69% of the sample from southern California and 53% of the sample from central California. A higher proportion of California halibut were older in southern California than in central California. Although California halibut can live as long as 30 years, the oldest fish found in our study was 13 years old. For southern California, the von Bertalanffy growth function (VBGF) was L_t=925.3(1–$e^{-0.08(t+2.2)}$) for males and L_t=1367.7(1–$e^{-0.08(t+1.2)}$) for females. For central California, the VBGF was L_t=956.7(1–$e^{-0.10(t+2.1)}$) for males and L_t=1477.1(1–$e^{-0.10(t+0.2)}$) for females. The VBGF showed that at the same age, females on average were larger than males in both southern and central California. The VGBF also showed that both male and female halibut in central areas on average were larger than halibut from southern California. Instantaneous mortality rates of halibut in southern California were estimated at 0.91 for males and 0.68 for females. Mortality estimates for central California could not be calculated because of small sample sizes.

California halibut, *Paralichthys californicus,* is an important flatfish species for sport and commercial fisheries in nearshore waters off central and southern California (Frey, 1971). The gear most commonly used commercially to harvest California halibut are otter trawls, gill nets, and trammel nets, whereas hook-and-line or spear are used mostly in the recreational fishery (Kramer and Sunada, 1992).

Information on age, growth, and mortality of California halibut are important to the management of both the sport and commercial halibut fisheries. Previous studies have addressed these issues to a limited extent. Pattison and McAllister (1990) studied techniques to age halibut and determined length-at-age of halibut along the California coast, but most of the fish were taken south of Point Conception and they did not do separate analyses for southern and central California. Sunada et al. (1990) analyzed age, size, and sex composition of commercial landings in southern California. Haaker (1975) studied the biology of California halibut in Anaheim Bay, including age and growth, but the fish he used were generally less than three years of age, immature, and included fish only to 510 mm total length. In contrast to other studies, our study provides information on age and growth for central and southern California separately. Mortality for southern California was also calculated.

Methods

Southern California was defined as the area between the U.S.-Mexico border and Point Conception, and central California as the area between Point Conception and Tomales Bay. Bottom trawl surveys were conducted off central California from 8 July through 3 August 1993 and off southern California from 14 February through 18 March 1994. California halibut were caught with a 400-mesh eastern trawl (15 m wide × 1.5 m high; 9.8 cm mesh body, 8.5 cm mesh codend). For both areas, sampling effort was stratified by depth: 0–20 fathoms (fm), 21–40 fm, and 41–60 fm. Fifty stations within each depth stratum were randomly selected for sampling by trawling parallel to isobaths at each station. The sample sizes were not proportional to the estimated area of the strata (Table 1).

An additional ten California halibut were collected with the same 400-mesh eastern trawl gear in southern California from July 1994 to June 1995. These were included in the age and growth determinations. However, they were not used for mortality estimates because they were collected at a different time period from the other halibut collected off southern California.

Each halibut was measured to the nearest millimeter for total length (TL) and standard length (SL). Gonads were examined visually to determine the sex of the fish. Sagittal otoliths were removed and stored dry in vials for age determination. Pattison and McAllister (1990) determined that when compared with other fish structures, otoliths provided the most reliable ages for California halibut. They found that in an otolith, an opaque band was formed during spring and summer (from April to October) and a translucent band was

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Table 1

Number of trawls and number of California halibut used in age and growth analyses by depth strata and region. Asterisk (*) indicates that these fish were not used in the mortality estimates because they were caught at a later date.

	Southern California				Central California		
Stratum	Depth (fm)	No. of trawls	No. of halibut used	Area (nmi^2)	No. of trawls	No. of halibut used	Area (nmi^2)
1	0–20	58	913 +3*	575	47	254	587
2	21–40	46	165 +2*	495	39	22	905
3	41–60	45	13 +1*	425	40	0	1124
Total			1091 +6*			276	

deposited in winter (from November to March). A combination of an inner opaque and adjacent outer translucent zone represents an annular growth ring or annulus (Casselman, 1983). This growth ring was validated by Pattison and McAllister (1990) with oxytetracycline-marked otoliths; they found that only one complete ring (opaque and translucent) was formed each year.

Pattison and McAllister suggested that age estimates of older fish (age 11 or older) may be underestimated when whole otoliths, rather than sectioned otoliths, are read. In the preliminary analysis in our study, to test if a significant difference existed between estimating age with whole otoliths and estimating age with sectioned otoliths, an otolith from each of 80 randomly selected fish (10 fish from each 100-mm size class from 200 to 900 mm TL) was viewed both whole and sectioned to estimate age. A mutually determined age (an age agreed upon by the two readers) was established for the whole otolith, as well as for the sectioned version of the otolith. If the age readings were identical for each reader, then that age was considered the correct age. Otherwise, the two readers discussed the readings and agreed upon an age. The nonparametric, paired Wilcoxon test was applied to the pairs of whole and sectioned otolith ages for each fish. In addition, a paired Wilcoxon test was applied to otoliths for fish restricted to 8 years and older (n=32).

In addition to determining whether to use whole or sectioned otoliths, the preliminary analysis also involved determining how halibut age was to be estimated: either by using the mutually determined age, as already described, or an averaged age. The averaged age was a single value calculated as the mean of the four independent readings (whole or sectioned version of an otolith read by two readers on two different occasions) and rounded to the nearest integer. Sample means generally have the same type of sampling distribution as individual observations, but with a smaller variance. Hence, we applied the nonparametric paired Wilcoxon test to the pairs of averaged and mutually determined ages for each otolith (n=80). The results from the preliminary analysis determined how we conducted the process of age determination in the main part of the study.

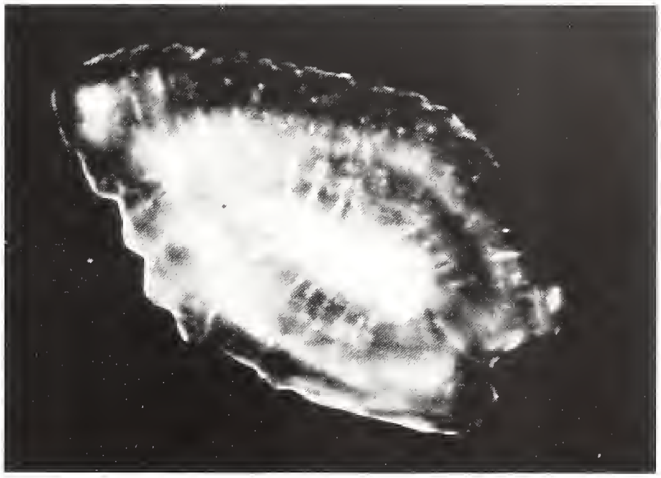

Figure 1

An example of an otolith viewed whole. Age was determined to be 3 years.

We acknowledge that using sectioned otoliths is the recommended procedure when analyzing long-lived fish. Although individual California halibut may live as long as 30 years (Frey, 1971; Pattison and McAllister, 1990), all the fish in our study were relatively young (less than 14 years old). Because of our preliminary analysis and because sectioning a large number of otoliths (potentially over 1300) would have entailed considerable costs, all otoliths were initially read whole. We did use sectioned otoliths when the variability in the age readings for that otolith seemed to be unacceptably high and when the whole otolith was judged unreadable because of the anise oil residue.

Before recording ages, two readers consulted with each other to standardize their technique for counting annuli. Each otolith was initially read whole, that is, the surface of the uncut otolith was read for the number of annuli present. The whole otolith was submerged in water, illuminated with fiber optic light transmitted from the sides, and viewed against a dark background with a dissecting microscope (16× to 25×). Figure 1 is an example of an otolith

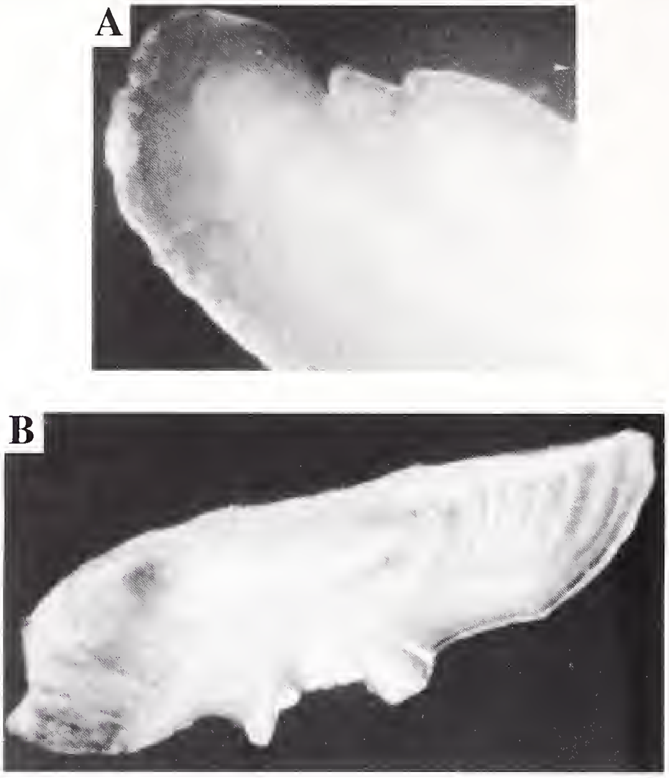

Figure 2
An example of a whole otolith that was unreadable (**A**) according to our criteria, but readable sectioned (**B**). Age was determined to be 9 years.

viewed whole. Some whole otoliths from central California were soaked in anise oil during initial handling to facilitate viewing annuli. The anise oil, however, left a residue that later caused problems in reading the otoliths. Therefore, these otoliths had to be sectioned before they could be read. For each otolith (either whole or sectioned), the two readers counted the annuli twice at different times, for a total of four independent readings. The birth date for a halibut was assigned to 1 January (Williams and Bedford, 1974).

The mean and standard deviation of the four age determinations for each whole otolith were calculated. Because of our preliminary analysis, if the standard deviation was less than 1.5 years, the mean, rounded to the nearest integer, was the estimated age for that halibut. A standard deviation of 1.5 years was arbitrarily chosen as the upper limit for acceptance of an age determination. This deviation represented a difference in ages of 3 years or more among readings. If the standard deviation was 1.5 years or larger the whole otolith was judged unreadable. These otoliths were cut laterally through the nucleus with a diamond blade on a Buehler low-speed Isomet saw. Three sections, approximately 0.38 mm thick, were cut from each otolith and mounted on a glass slide with Eukitt clear mounting medium. These sections were viewed with a compound microscope (25× to 100×) with transmitted light. The best of three sections on a slide was chosen for age determination. Figure 2 is an example of a whole otolith that was unreadable, but readable when sectioned.

The mean and standard deviation of the four age determinations for each sectioned otolith were calculated. In accordance with our preliminary analysis, if the standard deviation was less than 1.5 years, the rounded mean was considered the estimated age. If the standard deviation was 1.5 years or larger, the otolith was not used in the analysis because it was felt to be unreadable. The index of average percent error was calculated to compare within-reader precision (Beamish and Fournier, 1981) for both whole and sectioned otoliths.

Several analyses were made on total lengths and ages by sex and region. Comparisons of both length and age distributions between sexes for each region were made by using the two-sample Kolmogorov-Smirnov test (Hollander and Wolfe, 1973). Length-at-age data for females were compared with those for males sampled from the same region by using the nonparametric Mann-Whitney test. The Mann-Whitney test was also used to compare the length-at-age data between regions for each sex separately.

The von Bertalanffy growth function was fitted to the length-at-age data for individual fish (Ricker, 1975); parameters were estimated by nonlinear least squares by using the Gauss-Newton method in the program NLIN from SAS (1990). Outliers were removed when the von Bertalanffy growth function was fitted to the data. Outliers were data points that clearly stood apart in scatter plots of individual total length versus age. Means and standard deviations of observed length-at-age, and the theoretical von Bertalanffy growth function (VBGF) were plotted for females and males by region.

Hotelling's T^2 test (Bernard, 1981) compared growth parameters between male and female halibut in each region. This test was also used to determine if growth in southern California was different from growth in central California, for each sex separately.

The estimated annual survival rate, S, was calculated for males and females separately by region by using Robson and Chapman's method (1961). Total annual mortality, A, was calculated by using the complement of annual survival, $A = 1 - S$. The instantaneous mortality rate, Z, was estimated by using $-\ln(S)$ (Ricker, 1975). All statistical analyses were performed at the 0.05 level of significance.

Results

In our preliminary analysis, a subsample of whole otoliths from 80 fish were read and then sectioned to determine the best method for reading otoliths for age determination. In Figure 3 is a plot of the mutually determined ages from sectioned and whole otoliths, along with a 45 degree line and a linear regression. No significant difference was found between ages from whole and sectioned otoliths when using all 80 fish (paired Wilcoxon test, P=0.21), and when using only older fish (8 years and older) (paired Wilcoxon test, P=0.11).

Also, as part of the preliminary analysis, in the comparison of averaged ages with mutually determined ages for the estimation of halibut age, we found no significant differences for both whole (paired Wilcoxon test, mean difference=0.15, P=0.23) and sectioned ototliths (paired Wilcoxon test, mean difference=0.16, P=0.06). Therefore, ages were estimated for each individual by calculating an average of four independent readings from whole or sectioned otoliths because an average of several readings is considered a more reliable measurement than a single reading (Fleiss, 1986).

The total number of trawls conducted by strata and region are listed in Table 1. The original intent was to perform 50 trawls at each depth stratum for both southern and central California. However, substrate obstructions and other problems limited the number of successful tows at most strata. A total of 58 trawls were successfully completed in stratum 1 in southern California.

Because most of the halibut were collected in a single stratum (stratum 1), comparisons among strata were not made (Table 1). In southern California, a total of 916 individuals were collected from stratum 1 (84% of total), 167 individuals from stratum 2, and only 14 individuals from stratum 3. In central California, a total of 254 individuals were collected from stratum 1 (92% of total), 22 individuals from stratum 2, and none from stratum 3.

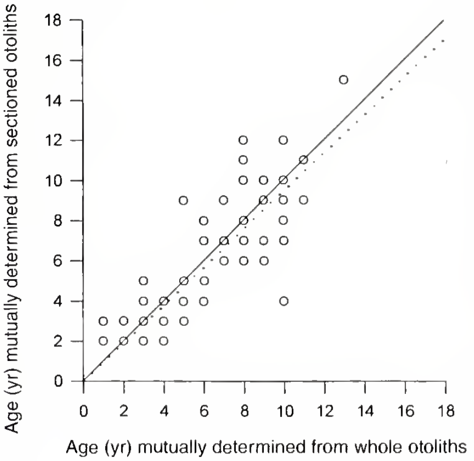

Figure 3

A scatter plot of mutually determined ages for sectioned otoliths and whole otoliths. The dotted line is the linear regression through the origin (slope is 0.947); solid line is the 45 degree line.

For southern California, pairs of whole otoliths from 1109 individuals were initially examined to estimate age. Twenty-two pairs of whole otoliths were unreadable, six pairs had no discernible opaque and translucent zones, and sixteen pairs had large variations in the four age readings (SD ≥1.5). These 22 pairs of whole otoliths were sectioned to determine age. Of these, two were eliminated because of large variations in the four age readings (SD ≥1.5). An additional ten were eliminated as outliers because lengths were anomalously small or large for the estimated age when the von Bertalanffy growth function was fitted to the data. Thus, a total of 1097 individuals were used to evaluate age and growth for halibut in southern California. The sample consisted of 69% males (761 individuals) and 31% females (336 individuals). Tables 2 and 3 provide counts of total length versus age for males and females, respectively, in southern California.

In central California, pairs of whole otoliths from 292 individuals were initially examined to estimate age. Otoliths from 28 halibut had no discernible opaque and translucent zones and 49 had large variations in the four age readings (SD ≥1.5), resulting in a total of 77 otoliths that were sectioned to estimate age. Of these, fourteen otolith pairs were eliminated because of large variations in readings (SD ≥1.5). An additional two became outliers that were removed when the von Bertalanffy growth function was fitted to the data. Thus, a total of 276 individuals were used to evaluate age and growth for halibut in central California. The sample consisted of 53% (147 individuals) males and 47% (129 individuals) females. Counts of total length versus age for males and females, respectively, in central California are shown in Tables 4 and 5.

Within-reader index of average percent error for whole otoliths ranged from 8.1% to 13.2%. For sectioned otoliths,

Table 2

Male California halibut total length versus age (mostly whole otoliths, some sectioned) in southern California.

	Age (yr)												
TL (mm)	1	2	3	4	5	6	7	8	9	10	11	12	Mean age
201–225		2	1										2.3
226–250	2	5	2										2.0
251–275	1	20	14										2.4
276–300	1	51	59	4	1								2.6
301–325		14	33	4	2								2.9
326–350		7	21	18	5								3.4
351–375		4	32	34	17	3							3.8
376–400			11	25	16	6	1						4.3
401–425			5	10	25	8	12	2					5.3
426–450				8	15	15	11	2					5.7
451–500				6	14	26	21	6	6				6.3
501–525				1	7	14	17	5	2				6.5
526–550					1	5	9	8	4	2			7.5
551–575					2	9	9	9	3	1			7.2
576–600					1	4	7	1	3	0	1	1	7.6
601–625						1	6	5	2	3			8.0
626–650						1	4	1	2				7.5
651–675													0.0
676–700													0.0
701–725								1	1				8.5
Mean length	259	289	319	377	417	482	506	538	552	578	577	586	

Median length of distribution: 387 mm TL

within-reader average percent error ranged from 4.7% to 8.9%.

In southern California, significant differences between males and females were found in comparisons of length distributions (Kolmogorov-Smirnov: D=0.42, P=0.0001). A higher proportion of males were smaller than females (Fig. 4). The median length for males was 387 mm TL (range: 210–707 mm TL); the median length for females was 544 mm TL (range: 241–987 mm TL) (Tables 2, 3, and 6). Females on average were significantly larger than males for ages 3 through 10 (Mann-Whitney tests, Table 6).

Some differences in lengths were also found between males and females in central California. Most of the larger fish were females (Fig. 4). However, the length distributions of males and females in central California were not significantly different (Kolmogorov-Smirnov: D=0.14, P=0.12). The median length for males was 437 mm TL (range: 285–781 mm TL); the median length for females was 444 mm TL (range: 262–1039 mm TL) (Tables 4, 5, and 7). Females on average were significantly larger than males for ages 3 and 5 through 8 (Mann-Whitney tests, Table 7).

In addition, comparisons were made between regions for each sex separately. In comparisons of mean length at ages, the males in central California on average were significantly larger than those in southern California for ages 2 through 9, except age 8 (Mann-Whitney tests, Table 8). The females in central California on average were significantly larger than those in southern California for only ages 5, 7, and 8 (Mann-Whitney tests, Table 8). However, the median length of females in southern California (544 mm) was greater than the median length for those in central California (444 mm).

In southern California, age distributions were significantly different for males and females (Kolmogorov-Smirnov: D=0.18, P=0.0001). A higher proportion of females were older fish compared with males. The primary age mode for females was age 6 and for males it was age 3 (Fig. 5). In central California, age distributions were also different for males and females (Kolmogorov: D=0.20, P=0.008). Males had a higher percentage in the 6 to 8 year range and a lower percentage in the 3 to 5 year range compared with females (Fig. 5). In central California, the primary mode for both males and females was age 3. With both regions combined, females were found up to age 13 and males up to age 12. In comparisons of age distributions between regions, we found that a higher proportion of halibut in southern California was older than halibut in central California (Fig. 5).

Because differences in length-at-age were found between sexes, von Bertalanffy growth parameters were cal-

Table 3

Female California halibut total length versus age (mostly whole otoliths, some sectioned) in southern California.

	Age (yr)													
TL (mm)	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean age
201–225														0.0
226–250		3												2.0
251–275	1	5	4											2.3
276–300		7	7											2.5
301–325		1	2											2.7
326–350		3	6	5										3.1
351–375		2	4	2										3.0
376–400			4	5	2									3.8
401–425			6	12	3	1								4.0
426–450			5	5	3	1								4.0
451–500			4	9	10	4	3	1						4.9
501–525				1	17	5	1							5.3
526–550				3	9	8	1	1	1					5.6
551–575				4	3	10	6	2						6.0
576–600				4	3	8	4	4	1					6.2
601–625				2	4	8	6	2						6.1
626–650					3	10	9	4	1					6.6
651–675				2	1	10	6	3	2					6.5
676–700						3	1	1	0	0	1			7.3
701–725							3	1	1	1				8.0
726–750						2	0	2						7.0
751–775							1							7.0
776–800								1	1	3				9.4
801–825								1	1	1				9.0
826–850								1	1	1				9.0
851–875								2	0	1				8.7
876–900														0.0
901–925									1	1				9.5
926–950									1	1				9.5
951–975													1	13.0
976–1000											1			11.0
Mean length	252	292	364	463	520	591	616	666	736	828	836	000	964	

Median length of distribution: 544 mm TL

culated separately for males and females for both regions (Table 9). Outliers were removed when the von Bertalanffy growth function was fitted to the data. With outliers removed, the standard errors of the asymptotic mean length, L_∞, became considerably smaller. Graphs of the von Bertalanffy growth function are shown in Figure 6 (where females and males are compared by region) and Figure 7 (where southern and central California are compared by sex).

Significant differences between males and females in southern California were found for all three growth parameters (Hotelling's T^2 tests, $P<0.0001$). For central California, two of the three growth parameters were significantly different between males and females (Hotelling's T^2 tests, $P<0.0001$). Females grew faster and on average were larger than males at the same age in both regions (Fig. 6).

Similar comparisons were made between regions for each sex separately (Fig. 7). For females, all three growth parameters were significantly different between southern and central California (Hotelling's T^2 tests, $P\leq0.003$). In contrast, the only significant difference between regions for males was in K, the Brody growth coefficient (Hotelling's T^2 tests, $P<0.0001$).

In southern California, survival rates were based on ages 7 to 12 years for males and ages 6 to 11 years for females. The estimated annual survival rates, S, for halibut from south-

Table 4

Male California halibut total length versus age (mostly whole otoliths, some sectioned) in central California.

	Age (yr)												
TL (mm)	1	2	3	4	5	6	7	8	9	10	11	12	Mean age
201–225													0.0
226–250													0.0
251–275													0.0
276–300		4	1										2.2
301–325		4	3										2.4
326–350		5	4										2.4
351–375			9										3.0
376–400		2	15	8									3.2
401–425			9	1	1								3.3
426–450			2	7	2								4.0
451–500			3	6	2	4	1	1					4.8
501–525				2	1	5	1	3					6.2
526–550				1	1	7	1	1					6.0
551–575					1	2	4	1					6.6
576–600					1	0	0	2					7.0
601–625							1	1	1				8.0
626–650						1	2	2	1				7.5
651–675				1	0	0	2						6.0
676–700						1	0	1	1				7.7
701–725								1					8.0
726–750							1	0	0	0	1		9.0
776–800										1			10.0
Mean length	000	326	385	448	493	539	596	582	649	781	737	000	

Median length of distribution: 437 mm TL

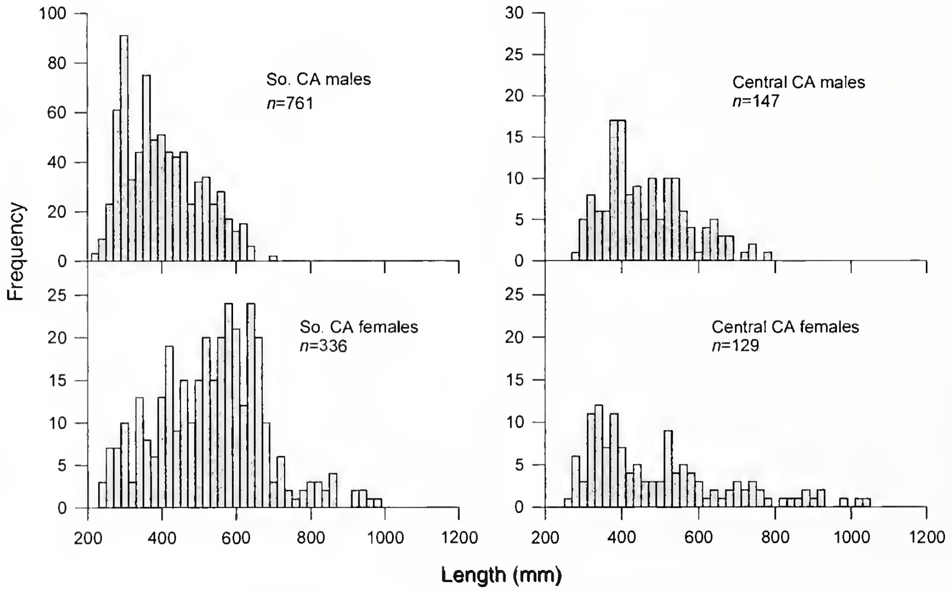

Figure 4

Length frequencies for male and female California halibut sampled off southern California (So. CA) and central California (Central CA).

Table 5

Female California halibut total length versus age (mostly whole otoliths, some sectioned) in central California.

	Age (yr)													
TL (mm)	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean age
201–225														0.0
226–250														0.0
251–275		1												2.0
276–300		3	4											2.6
301–325		5	7											2.6
326–350		2	10	2										3.0
351–375		1	7											2.9
376–400			8	5										3.4
401–425			3	2										3.4
426–450			2	6										3.8
451–500			1	3	2									4.2
501–525				7	2									4.2
526–550			2	1	3									4.2
551–575			1	3	1									4.0
576–600			1	2	2	1								4.5
601–625				1										4.0
626–650					1	1	0	0	1					6.7
651–675				1	1									4.5
676–700				1	0	1	1	1						6.3
701–725					1	0	0	1						6.5
726–750					3									5.0
751–775					1	0	1							6.0
776–800						1								6.0
801–825				1										4.0
826–850								1						8.0
851–875									1					9.0
876–900								1	1					8.5
901–925								1	2					8.7
926–950														0.0
951–975										1				10.0
976–1000														0.0
1001–1025												1		12.0
1026–1050												1		12.0
Mean length	000	313	373	493	606	672	726	810	842	970	000	1027	000	

Median length of distribution: 444 mm TL

ern California were 0.40 (95% confidence interval=0.34, 0.46) for males and 0.51 (95% confidence interval=0.45, 0.57) for females. Estimated annual mortality rates, A, were 0.60 for males and 0.49 for females. Instantaneous mortalities, Z, were estimated at 0.91 for males and 0.68 for females. We were unable to calculate survival rates and mortalities for central California halibut because the sample sizes were too small.

Discussion

The California halibut can be a long-lived species, living as long as 30 years (Frey, 1971; Pattison and McAllister, 1990). However, none of the fish in our study was older than 13 years, and most of the fish were less than 11 years old (Fig. 5). We feel that the low average percent error in our study showed that both whole and sectioned otoliths were reliable methods for aging California halibut for the age range of our study.

Pattison and McAllister (1990) suggested that because of the asymmetrical growth of the sagittae in larger fish, ages assigned to older fish (age 11 or older) may be underestimated if whole otoliths, rather than sectioned otoliths, were read. Manooch and Potts (1997) in a growth study of greater amberjack also did not find whole otoliths useful in aging fish. In contrast, we found that age estimates from whole and sectioned otoliths were not significantly different, even when the analysis was limited to ages 8 to 13 years. However, our conclusions are limited to primarily younger fish because most of the fish in our study were less than 11 years old. Pattison and McAllister recommended sectioned otoliths for fish aged 11 years or older. Nonetheless, we concur that use of sectioned otoliths in older individuals may reduce the variation in assigned ages.

We feel that the larger percentage of unreadable whole otoliths in central California versus southern California was due primarily to the preparation techniques used for age determination. The anise oil used in the initial handling of some central California otoliths created a white film that precluded the otoliths from being read at a later time. Because of problems encountered with anise oil, we do not recommend applying it to otoliths until one is ready to age them.

Table 6

Mean total length (mm) and standard deviation for male and female California halibut by age group in southern California. Mann-Whitney comparisons of male and female halibut for mean length at age. Asterisk (*) indicates statistical significance.

Age (yr)	Male *n*	Male Mean	Male SD	Male Min	Male Max	Female *n*	Female Mean	Female SD	Female Min	Female Max	Mann-Whitney *P*-value
1	4	259.3	25.0	232	290	1	252.0	—	252	252	—
2	103	289.4	28.4	210	369	21	291.9	38.4	241	368	0.77
3	178	319.3	39.3	220	411	42	364.4	63.9	254	477	0.0001*
4	110	377.3	42.2	291	501	54	462.6	86.7	342	660	0.0001*
5	106	417.2	55.0	294	561	58	520.5	64.3	389	671	0.0001*
6	92	481.7	61.9	362	646	70	591.2	67.7	401	735	0.0001*
7	97	505.6	65.1	399	645	41	616.1	60.5	493	758	0.0001*
8	40	538.3	62.6	403	707	26	666.0	101.4	492	859	0.0001*
9	23	551.5	68.0	451	707	11	736.2	131.9	534	934	0.0002*
10	6	578.2	34.5	542	619	9	828.3	67.4	720	937	0.002*
11	1	577.0	—	577	577	2	836.0	213.5	685	987	—
12	1	586.0	—	586	586	—	—	—	—	—	—
13	—	—	—	—	—	1	964.0	—	964	964	—
Total	761					336					

Table 7

Mean total length (mm) and standard deviation for male and female California halibut by age-group in central California. Mann-Whitney comparisons of male and female halibut for mean length at age. Asterisk (*) indicates statistical significance.

Age (yr)	Male *n*	Male Mean	Male SD	Male Min	Male Max	Female *n*	Female Mean	Female SD	Female Min	Female Max	Mann-Whitney *P*-value
2	15	325.9	29.8	285	381	12	312.9	28.7	262	367	0.29
3	46	384.8	38.1	293	472	46	373.4	70.0	279	593	0.02*
4	26	447.8	60.5	380	660	35	493.5	102.3	328	820	0.07
5	9	492.8	59.0	424	582	17	606.2	101.4	474	758	0.007*
6	20	538.6	49.4	479	680	4	672.0	87.0	582	782	0.006*
7	13	595.6	72.7	490	748	2	725.5	43.1	695	756	0.05*
8	13	582.5	74.4	475	719	5	810.4	99.3	694	925	0.002*
9	3	648.7	29.5	619	678	5	842.0	112.2	547	923	—
10	1	781.0	—	781	781	1	970.0	—	970	970	—
11	1	737.0	—	737	737	—	—	—	—	—	—
12	—	—	—	—	—	2	1026.5	17.7	1014	1039	—
Total	147					129					

The data in our study confirmed that females grow faster than males, as found in previous studies (Pattison and McAllister, 1990; Sunada et al., 1990), and that growth was different between males and females for each region. Females in both regions had larger asymptotic mean lengths, L_∞, than males (Table 9).

Growth curves for males and females crossed between ages 1 and 2 for southern California and at age 3 for central California (Fig. 6). This finding suggests that young male and female halibut are of similar size until about ages 2 and 3 in southern and central California, respectively. However, Haaker (1975) found that juvenile female halibut in Anaheim Bay grew faster than males. The crossover in growth curves may be due to the small sample sizes for ages 1 and 2 in our study. The growth curves also involved some extrapolation for very young ages.

Our study showed lower mean length-at-age than that obtained by Pattison and McAllister (1990); however, the differences for females were slight. These differences may partly be attributed to different environmental conditions in the years prior to each study. Pattison and McAllister used otoliths collected from 1955–66 and 1984–88, whereas we used otoliths collected from 1993 to 1995. Manooch and Potts (1997) in a study of greater amberjack in the Gulf of Mexico also cited temporal changes as one of the factors that may have contributed to differences in growth between greater amberjack in southern Florida and northern Gulf of Mexico. The geographic range of the samples may also have affected the results because we found growth differences between southern and central California. Pattison and McAllister's study encompassed samples from central and southern California, but they did not separate the two regions in their analysis. In addition, gear selectivity may have contributed to the differences found between our two studies. Pattison and McAllister used halibut that were collected with several gear types (trawl, gill net, beach seine, hook and line, and spear), whereas our study sampled halibut with only trawl gear of a specific mesh size. Potts et al. (1998) found while studying vermillion snapper from the southeastern United States that the different gear types used in sample collection may bias growth results.

Sunada et al. (1990) found that for commercial halibut landings in southern California the asymptotic mean length, L_∞, was 909 mm TL for males and 1445 mm TL for females. In comparison, our study showed for southern California a slightly higher L_∞ for males at 925 mm TL, and a lower L_∞ for females at 1368 mm TL. The asymptotic mean length for females may be lower because fewer large females were collected in our study. Although our study had different growth parameters than those of Sunada et al. (1990), the differences were only slight. The differences may have been due to gear vulnerability. Sunada et al. (1990) collected halibut from both trawl nets and gill nets and found that fewer females were collected in trawl nets, which may be attributed to trawl gear being restricted to offshore areas during spawning season. Also

Table 8

Mann-Whitney comparisons of California halibut from southern and central California for mean length at age. Asterisk (*) indicates statistical significance.

Age (yr)	Mean observed total length (mm) Southern	Mean observed total length (mm) Central	Sample size n Southern	Sample size n Central	Mann-Whitney P-value
Males					
2	289.4	325.9	103	15	0.0001*
3	319.3	384.8	178	46	0.0001*
4	377.3	447.8	110	26	0.0001*
5	417.2	492.8	106	9	0.0006*
6	481.7	538.6	92	20	0.0002*
7	505.6	595.6	97	13	0.0002*
8	538.3	582.5	40	13	0.08
9	551.5	648.7	23	3	0.03*
Females					
2	291.9	312.9	21	12	0.10
3	364.4	373.4	42	46	0.92
4	462.6	493.5	54	35	0.21
5	520.5	606.2	58	17	0.002*
6	591.2	672.0	70	4	0.07
7	616.1	725.5	41	2	0.04*
8	666.0	810.4	26	5	0.01*
9	736.2	842.0	11	5	0.17

Table 9

Von Bertalanffy growth parameters with asymptotic standard errors (SE) for California halibut by sex in southern and central California. Length is given in mm TL.

Region	Sex	n	L_∞	SE	K	SE	t_0	SE
Southern	male	761	925.3	121.4	0.08	0.02	–2.2	0.41
	female	336	1367.7	273.4	0.08	0.03	–1.2	0.48
Central	male	147	956.7	211.9	0.10	0.05	–2.1	0.91
	female	129	1477.1	308.1	0.10	0.04	–0.2	0.43

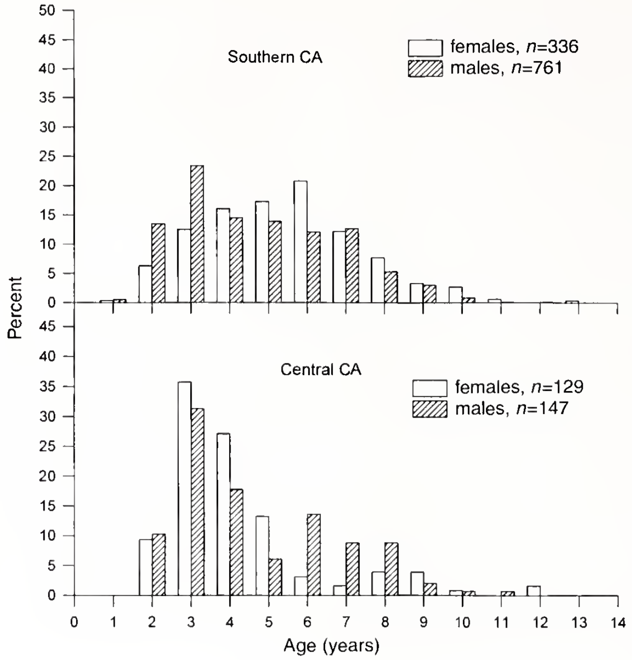

Figure 5

Age frequencies in percent for male and female California halibut sampled off southern and central California (CA).

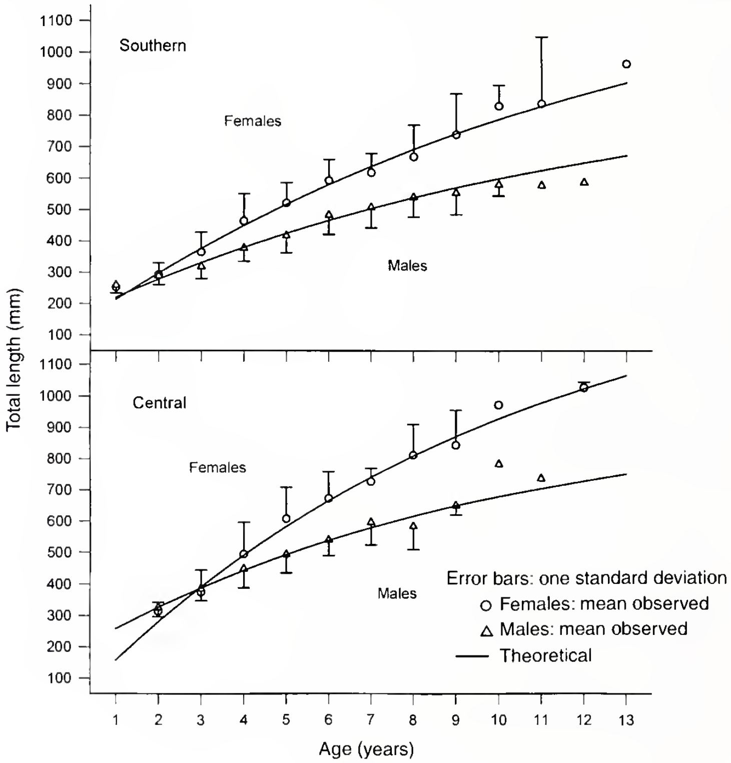

Figure 6

Von Bertalanffy growth curves for comparing growth between sexes by region.

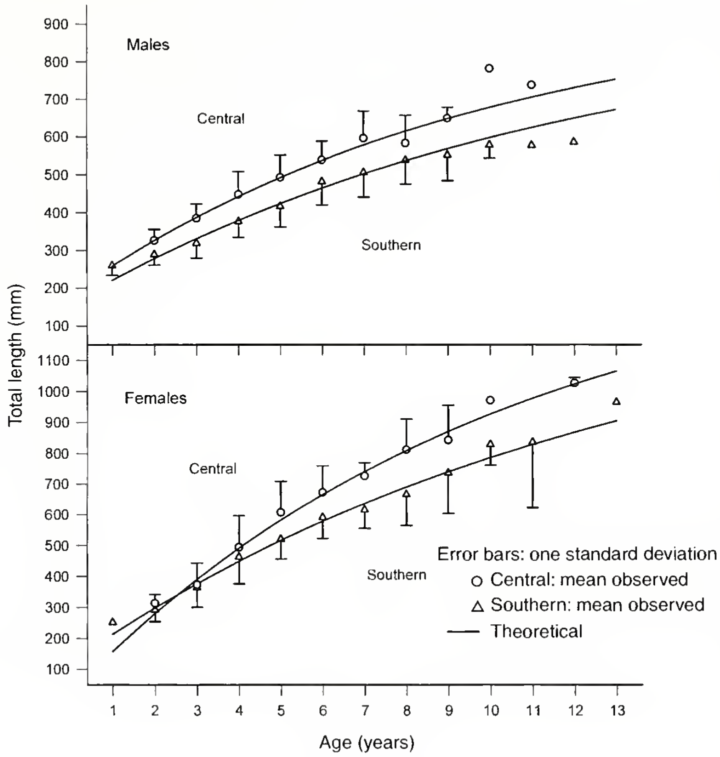

Figure 7

Von Bertalanffy growth curves for comparing growth between regions by sex.

females, which are generally larger, may be more capable of escaping a trawl net than males. Other factors that may have affected the growth parameters include the following: method of collection, time periods of sampling, and different environmental conditions in the years prior to each study.

Many factors can affect growth rates of fish including differences in the seasonality of spawning, environmental factors, amount and size of food, and genetics (Weatherly and Gill, 1987; Moyle and Cech, 1988). Southern and central California are biogeographically different. Differences between central and southern California coastal waters include temperature, water circulation patterns, bottom topography, and substrate. In particular, Jow (1990) noted differences in bottom topography and substrate between the trawl areas for California halibut in southern and northern (San Francisco Bay area) California. The biogeographical differences among these regions could possibly cause growth rates of the same species to differ. Studies of other fish species have shown differences in growth between geographical regions. For example, Parrish et al. (1985) found latitudinal differences in the growth of northern anchovy, *Engraulis mordax*. They found that the growth rate of juvenile northern anchovy in central California was greater than that of juvenile northern anchovy in southern California. Butler et al. (1996) found differences in maturation and length at age of Pacific sardine between latitudinal regions. Sardine appeared to mature at a younger age off both southern and Baja California than off Monterey; one-, two-, and three-year old fish were smaller at age off Baja and larger off Monterey. Deriso et al. (1996) confirmed the findings of sardine growth differences by Butler et al. (1996). The exact mechanism for latitudinal differences in growth rates of California halibut is still unclear and further research is needed to clarify this phenomenon.

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Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae)

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Abstract—Morphological development and growth of larval and juvenile Pacific bluefin tuna, *Thunnus thynnus*, were studied from laboratory-reared specimens. Average body length (BL) of newly hatched larvae was 2.83 mm and larvae grew on average 5.80 mm by 10 days, 10.62 mm by 20 days, and 35.74 mm by 30 days after hatching. Growth was especially accelerated after 20 days from hatching. Newly hatched larvae had small melanophores scattered over their bodies except for the finfold. On day 1 after hatching (3.35–3.74 mm BL), a characteristic melanophore pattern appeared and it was partially maintained until day 3 after hatching. At approximately 4 mm BL, larvae had developed melanophore patterns similar to those of preflexion *Thunnus* spp. larvae, such as melanophores on the dorsum of the gut, midlateral trunk, and tail, at the dorsal and ventral midlines of trunk and tail, and on the lower jaw. Erythrophores appeared at 4.63 mm BL in the caudal area. Jaw teeth appeared at 5–6 mm BL. The preopercular angle spine, anterior preopercular spine, and posttemporal spine, developed at approximately 7 mm BL, when erythrophores appeared on the trunk and tail. The notochord flexion occurred between 6 and 8 mm BL. At approximately 8 mm BL, erythrophores disappeared and juvenile coloration appeared on the trunk and tail, consisting of dense patches of melanophores at the dorsal and anal-fin bases, embedded melanophores, and melanophores at the periphery of the eye. The adult complement of fin-ray counts was attained at 10 mm BL, when the juvenile melanophore pattern was attained, although the pattern was not fully developed. Specimens larger than 20 mm BL did not have erythrophores. Squamation began at 27 mm BL and head spines disappeared by 38 mm BL.

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The Pacific bluefin tuna is a large scombrid that migrates transoceanically between the western and eastern Pacific Ocean (Orange and Fink, 1963; Fujita, 1998). The Pacific bluefin tuna, *Thunnus thynnus orientalis* (Temminck and Schlegel), is considered a separate subspecies from the Atlantic bluefin tuna, *T. thynnus thynnus* Linnaeus (Gibbs and Collette, 1967; Collette and Nauen, 1983). Recently, Cho and Inoue (1993) showed reproductive isolation between the two subspecies. Most recently, Collette (1999) considered these subspecies to be separate species on the basis of morphology and molecular data.

Our knowledge of the early life history of oceanic species, such as tunas, is incomplete. Morphological characteristics common to scombrid larvae are large head, gape, and eyes; development of head spination; and posterior migration of the anus (Collette et al., 1984). In studies of early-stage bluefin tuna (e.g. Yabe et al., 1966; Matsumoto et al., 1972; Richards and Potthoff, 1974; Kohno et al., 1982), melanophore patterns were used to identify tuna larvae. Erythrophore patterns were proposed as another characteristic by Ueyanagi (1966) and Matsumoto et al. (1972). However, ontogenetic changes of melanophore and erythrophore patterns and morphological characteristics have not sufficiently been investigated because of the difficulty in obtaining a complete series of wild specimens in their early developmental stages.

Morphological, physiological, and behavioral information has recently been collected from laboratory-reared Pacific bluefin tuna specimens (Harada et al., 1971a; Kaji et al., 1996; Takii et al., 1997; Kumai, 1998; Miyashita et al., 1998; Miyashita et al., 2000, in press; Sawada et al., 2000). The ability to produce a complete series of early-stage specimens provides an opportunity to enhance our understanding of the early development of bluefin tuna. In this study, we describe the morphological development and pigment patterns of reared larval and juvenile bluefin tuna.

Materials and methods

Eggs were obtained on 10 July 1994 from bluefin tuna that had been caught off Ohshima when 20–40 cm in total length [0-year class] and that had been raised for seven years naturally in a net cage at the Ohshima Experiment Station of the Kinki University Fisheries Laboratory. The net cage was equipped with a polyvinyl chloride sheet surrounding the top 2 m of the net side wall to prevent eggs from flowing out of the net. Fertilized eggs were collected at the surface with a 330-µm mesh net. Eggs were transferred to a tank in a land-based nursery center and kept in a fine mesh net at 24.5°C for approximately 12 hours. The following morning, eggs were transferred to a 20-m^3 rearing tank and larvae hatched at approximately 2200 h on 11 July. The first 24 hours after hatching were counted as day 0 (day-0) after hatching. Water temperature ranged from 24.5 to 27.7°C during the rearing period ($\bar{x}$=25.5°C, Fig. 1). The feeding scheme for larvae and juveniles was as follows: rotifers, *Brachionus rotundiformis,* from day 2 to day 22 after hatching, *Artemia* nauplii from day 10 to day 25, and other live fish larvae (*Oplegnathus fasciatus*) from day 12 to day 30.

At 1000 h daily from day 0 (12 July) to day 30 (11 August), 20 tuna were sampled. Before preservation in 5% formalin, individual computer-captured video images were made with a video camera attached to a binocular microscope while all specimens were under MS-222 anesthesia. Ten body parts were measured from these images: total length (TL), body length (BL), preanal length, body height at the base of the first dorsal spine, head length, head height, snout length, eye diameter, caudal peduncle depth, and upper jaw length. Video image measurements to $1/10^2$ mm with an accuracy of ±1% were obtained from NIH Image (version 1.61) image analysis software (NIH, 1997). Before and during notochord flexion, BL was measured from the tip of the upper jaw to the end of the notochord. After notochord flexion, BL was measured from the tip of the upper jaw to the posterior margin of the hypurals. All specimens (n=620, 3.49–37.78 mm BL) were used to determine growth estimates and to record pigmentation patterns and spine and fin development.

Sixty-nine specimens (5.26–33.68 mm BL) were stained with alizarin red-S to describe spine development, and squamation. Pigment patterns, especially the position of body melanophores in relation to myomeres, were determined for specimens from 3 to 16 days after hatching (n=180, 3.63–9.96 mm BL). At irregular intervals from day 0 to day 22, one to five fish (a total of 53 specimens, 3.38–26.76 mm BL) were examined while anesthetized with MS-222 to determine erythrophore distribution. Additional samples were examined at irregular intervals from hatching to day 2.

A representative series of specimens from this study was deposited in the Aquatic Natural History Museum of the Fisheries Research Station, Kyoto University (FAKU 129041–129075).

Results

Structural characteristics and growth

Eggs, having a mean diameter of 1.02 mm (SD=0.02; n=20), were spherical and pelagic and had a single oil globule of 0.26 ±0.05 (mean ±SD) mm in diameter.

Newly hatched larvae measured 2.83 ±0.16 mm in BL (mean ±SD, n=20; Fig. 2A). At 3 days after hatching (3.81 ±0.14 mm BL), the mouth was developed and larvae began feeding. Yolk was present until day 5 (4.32 ±0.13 mm BL). Larvae grew to 5.58 ±0.35 mm by day 10, 9.34 ±1.36 mm by day 20 and 30.36 ±4.34 mm by day 30 after hatching (Fig.1). From hatching to day 20, growth rate was 0.33 mm/day, then increased to 2.10 mm/day from day 20 to day 30.

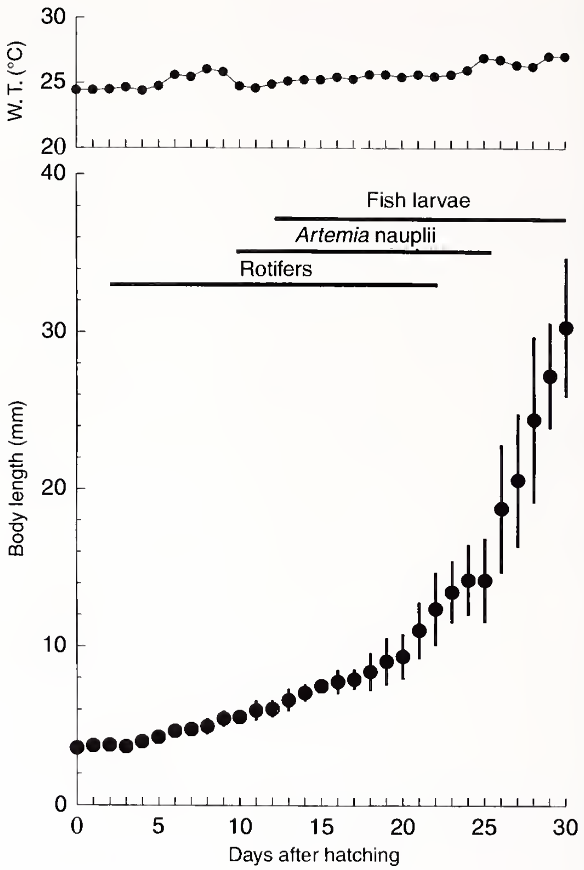

Figure 1

Body length, diet, and water temperature (W.T.) during the rearing of *Thunnus thynnus*. Vertical lines indicate ±SD (n=20).

Notochord flexion began as early as 5.76 mm BL, and the largest preflexion larva was 5.83 mm BL. The smallest postflexion larva was 7.46 mm BL; the largest flexion larva, 8.65 mm BL. Thus, the flexion stage occurred from 6 to 8 mm BL (Fig. 2E).

The relative values of the nine body parts, except preanal length, increased during larval development, then subsequently declined (Fig. 3). The ratio of head length, head height, eye diameter, snout length, and upper jaw length to BL reached a maximum at 11–13 mm BL. Head length increased rapidly from 10% BL at hatching to 40% at 13 mm BL and then decreased slowly to 34 % BL at 40 mm BL. Head height also increased rapidly from 10% BL at hatching to 27% at 11 mm BL and decreased slowly to 23% BL at 40 mm BL. Eye diameter increased from 10% BL at hatching to 15% at 11 mm BL and then decreased slowly to 9% BL at 40 mm BL. Snout length rapidly increased from 2% BL at hatching to 14% at 12 mm BL and then decreased slowly to 9% BL at 40 mm BL. Upper jaw length increased rapidly from 5% BL at hatching to 20–30% at 12 mm BL and then decreased slowly to 14% BL at 40 mm BL. The

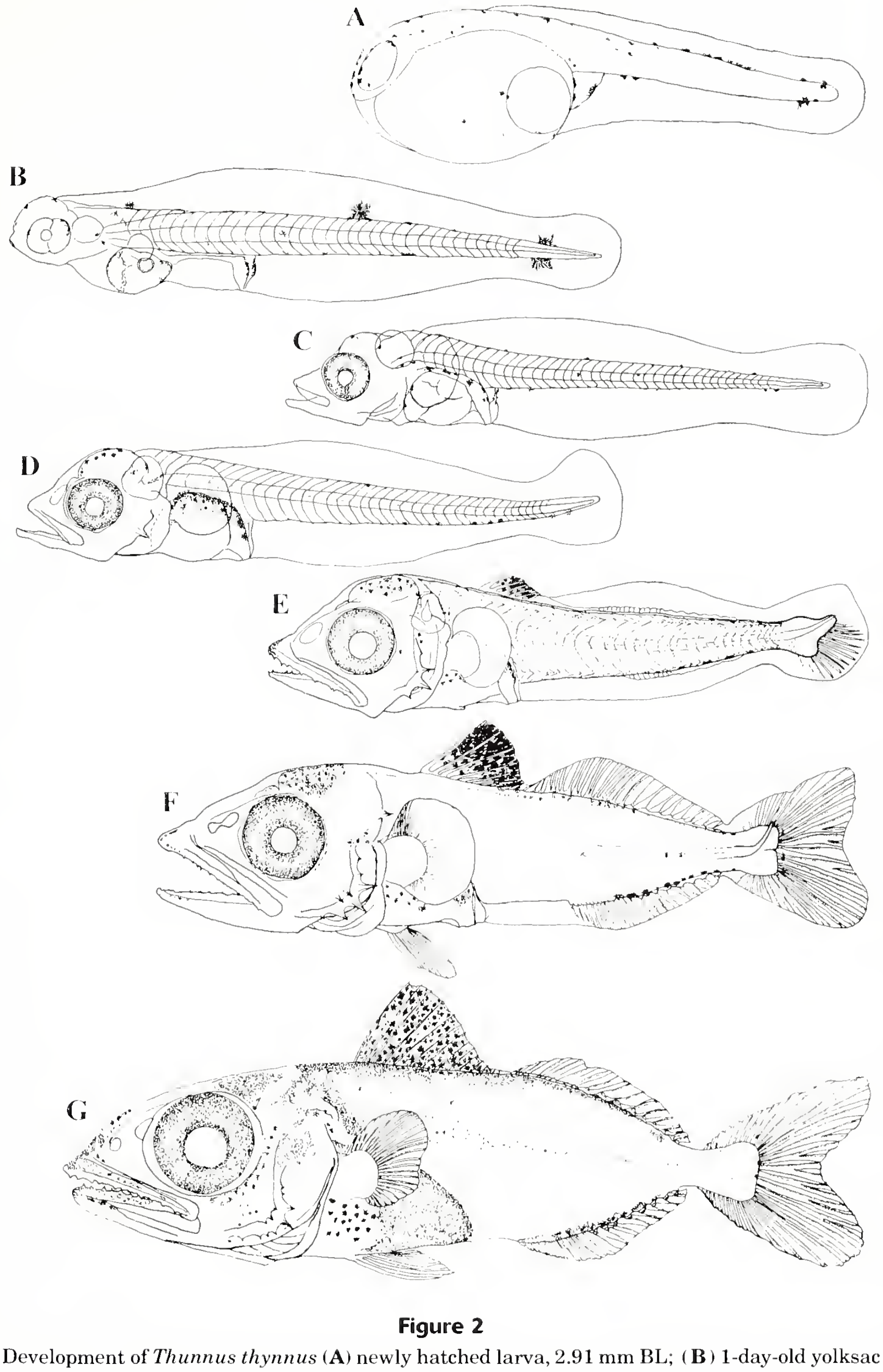

Figure 2

Development of *Thunnus thynnus* (**A**) newly hatched larva, 2.91 mm BL; (**B**) 1-day-old yolksac larva, 3.54 mm BL; (**C**) 4-day-old preflexion larva, 4.02 mm BL; (**D**) 7-day-old flexion larva, 5.34 mm BL; (**E**) 15-day-old postflexion larva, 7.23 mm BL; (**F**) 20-day-old postflexion larva, 8.64 mm BL; (**G**) 25-day-old juvenile, 13.55 mm BL.

relative depth of the caudal peduncle attained a maximum (ca. 7% BL) at 8 mm BL. In contrast, body height and total length were maximum in relation to BL at approximately 16 and 20 mm BL, respectively. Preanal length increased from 35–44% BL at 3–7 mm BL to about 65% BL at approximately 15 mm BL.

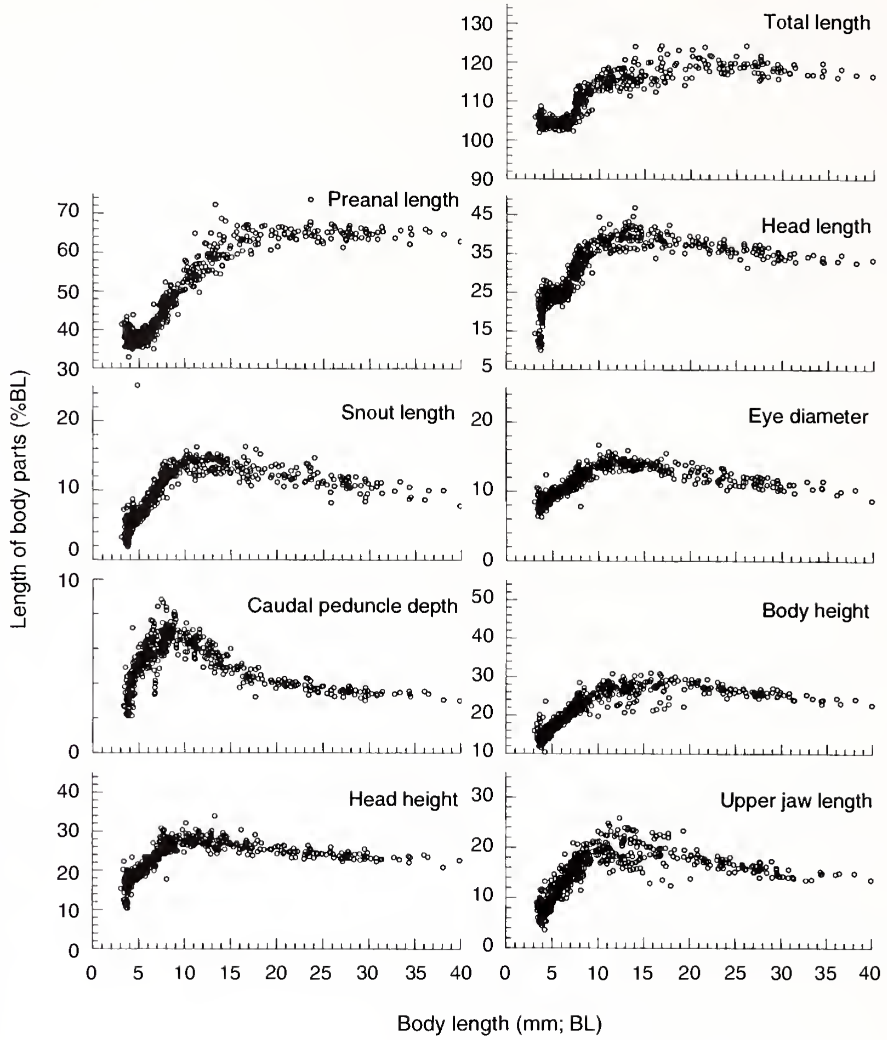

Figure 3

Measurements of *Thunnus thynnus*, shown as a percentage of body length (BL).

Table 1

Numbers of fin spines and rays of *Thunnus thynnus* larvae and juveniles. Roman and Arabic numerals show numbers of spines and soft rays, respectively.

Size range (BL; mm)	First dorsal fin	Second dorsal fin	Dorsal finlet	Anal fin	Pectoral fin	Pelvic fin	Number of specimens examined
BL ≤5.99	0	0	0	0	0	0	10
6.00–6.99	I–X	0	0	0–6	0–13	0–I+1	17
7.00–7.99	0–XIV	0–13	0–8	0–12	0–31	0–I+5	13
8.00–8.99	IX–XIV	12–14	6–9	7–14	11–31	I+4–I+5	11
9.00–9.99	XI–XV	14	8–9	13–14	16–31	I+5	8
10.00–19.99	XIV–XV	14	8–9	14	31–34	I+5	14
20.00–29.99	XV	14	8–9	13–14	31–36	I+5	19
30.00–37.78	XV	14	8–9	14	31–35	I+5	8

Fin development

The number of fin rays increased between 6 and 10 mm BL (Table 1). The smallest specimen possessing dorsal spines was 6.32 mm BL. Caudal-fin rays, second dorsal-, pelvic-, anal-, and pectoral-fin rays appeared at 7.10, 7.76, 8.00, 8.47, and 8.67 mm BL, respectively. The smallest specimen with an adult complement of fin-ray counts (Iwai et al., 1965; Collette and Nauen, 1983) was 9.68 mm BL, whereas the largest specimen with an incomplete fin-ray complement was 10.02 mm BL. The number of pectoral-fin rays for specimens >10 mm BL varied among individuals.

Pigmentation

Newly hatched larvae (2.62–3.05 mm BL) had small melanophores scattered over the body, head, notochord, yolk, and oil globule, but not on the finfold (Fig. 2A).

On day 1 after hatching (3.35–3.74 mm BL) (Fig. 2B), dendritic melanophores were visible from snout to fore- and midbrain and on the dorsum of the gut; three clusters of melanophores occurred in the dorsal midline along with punctate melanophores; melanophores occurred along the ventral midline (punctate melanophores formed a line at the anterior caudal area and a cluster of melanophores occurred in the posterior caudal area); and melanophores appeared along the lateral midline of the trunk. The eyes were pigmented.

On day 2 after hatching (3.40–4.18 mm BL), the pattern of melanophores was transitional between those of 1-day and older preflexion larvae. Melanophores on the snout disappeared, whereas clusters of melanophores on the anteriodorsum of the trunk (second myomere), anteriordorsum of the caudal area (17th to 21st myomere) and posteriodorsum of the caudal area (30th to 39th myomere), as well as on the anterio- and midventral edge (14th to 26th myomere), posteroventral edge (28th to 39th myomere), and lateral midline (posterior to the 13th myomere) of the caudal area began to shrink (although they were still large [Tables 2–5]).

On day 3 after hatching, forebrain melanophores disappeared in all specimens >3.76 mm BL, whereas the smallest specimen lacking these melanophores was 3.63 mm BL. All specimens >3.82 mm BL had melanophores scattered on the dorsum of the gut, which formed a cap of melanophores over the gut in later stages. Clusters of melanophores on the dorsal and ventral midline, and lateral midline of the body now appeared punctate and additional punctate melanophores appeared in these areas (Tables 2–5). Melanophores were present on the midbrain, on the anteriodorsal side and on the base of the hindbrain; and several melanophores appeared on the lower side of the lower jaw. The smallest specimen with melanophores on the lower jaw was 3.41 mm BL, whereas the largest lacking them was 9.46 mm BL (day 16). Most specimens >5.5 mm BL had these lower jaw melanophores. On and after day 4 (3.92–4.43 mm BL), all specimens had the melanophore pattern of preflexion larvae (Fig. 2C).

Melanophores began to develop on several other areas in larvae larger than about 4.5 mm BL (Fig. 2, D–F). Embedded melanophores appeared in the lateral muscle at 4.49 mm BL (Table 6), upper jaw tip at 5.47 mm BL, operculum and preoperculum at 6.18 mm BL, the membrane of the first dorsal fin at 6.32 mm BL, forebrain at 6.83 mm BL, and the cleithral symphysis at 10.79 mm BL. The largest specimens lacking melanophores in these areas were those that were 6.44 mm BL (for upper jaw tip), 6.72 mm BL (for lower jaw tip), 6.94 mm BL (for operculum), 9.69 mm BL (for forebrain), 10.28 mm BL (for cleithral symphysis), and 10.49 mm BL (for preoperculum).

Melanophores forming a dorsal cap of the gut enlarged at about 6.5 mm BL and the rim reached the ventral surface of the gut in some specimens >7.5 mm BL. The body melanophore pattern changed with growth. Dorsal midline melanophores disappeared from the trunk from 4.5 mm BL to 7 mm BL, and ventral and lateral midline melanophores disappeared from the trunk (Tables 2–5).

Larvae showed partial juvenile pigment patterns from about 8 mm BL (Fig. 2F). Dorsolateral and dorsal midline melanophores appeared at the trunk and increased in frequency in the caudal area from 7.5 mm BL (Table 2). Embedded melanophores frequently appeared at the posterior trunk and caudal area (Table 6). Dense patches of melanophores appeared at the fin base of the first and second dorsal, and anal fin bases (Tables 7 and 8; these melanophores occurred on the myosepta), as well as at the periphery of the eye, especially below and behind the eye from about 8.0 mm BL. Embedded melanophores appeared near the notochord and neural and haemal spines, and melanophores at the lateral midline of body extended internally from about 8.2 mm BL. Generally, melanophores on the dorsal and ventral midline, lateral midline, and those embedded increased with BL (Tables 2–6).

The smallest specimen having juvenile pigmentation (i.e. densely pigmented patches appearing on the body) was 13.55 mm BL (Fig. 2G). More distinct body patches appeared and increased in number as juveniles grew.

In many larvae, melanophores were found in the area of the hypural bones and on the caudal finfold or fin rays. The smallest specimens having melanophores in the area of hypural bones and on the caudal finfold were 6.42 mm BL and 6.81 mm BL, respectively.

Erythrophores first appeared at 4.63 mm BL (6 days after hatching) on the tail: thirty-four erythrophores at the posteroventral edge, five at the posterolateral midline, and two at the posterodorsal edge (Table 9). As larvae grew, erythrophores appeared at the caudal finfold at 4.75 mm BL, on the lower jaw at 6.10 mm BL, and at the hypural plate at 6.96 mm BL; at the hypural plate and caudal finfold, erythrophores were observed only on specimens 6.96–7.20 and 4.75–7.20 mm BL, respectively. At the dorsal and ventral edge of the trunk and tail, erythrophores became larger and decreased in number in larvae >7 mm. At the ventral edge, adjacent erythrophores were united. At the lateral midline of the body, the number decreased at 8.5 mm BL. Erythrophores disappeared from the lower jaw at 11.26 mm BL, from the posterolateral midline and dorsal edge of the tail at 15.85 mm BL, and from the ventral edge of the tail at 19.72 mm BL. Erythrophores were not observed on the upper jaw. The number of erythrophores, especially the ventral edge erythrophores, de-

Table 2

Incidence of dorsal midline melanophores at the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is

Size range (BL; mm)	Myomere 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3.50–3.99		14					7					14	7	7	7	7	36	29	36
4.00–4.49		4	4							4	15		4	4	7	7	15	41	22
4.50–4.99					4	4									4	4	7	15	7
5.00–5.49											8				8	8	8		17
5.50–5.99		10	10											10	10	10			
6.00–6.49																	20	30	30
6.50–6.99																			17
7.00–7.49											10	10	10	10	20	20		30	
7.50–7.99										10	10	10	20	40	20	40	20	10	20
8.00–8.49							8	15	31	23	62	77	77	85	85	85	69	69	62
8.50–8.99	23	54	38	46	46	54	54	54	62	85	85	85	85	92	85	92	92	92	92
9.00–9.49	25	67	17	25	33	33	83	58	50	58	58	67	67	67	58	75	83	67	67
9.50–9.99	30	80	20	30	40	40	100	70	60	70	70	80	80	80	70	90	100	80	80

Table 3

Incidence of the ventral midline and ventrolateral melanophores of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore not observed on the first to sixth myomeres.

Size range (BL; mm)	Myomere 6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
3.50–3.99	7	7				7	7	7	14	14	29	7	7	50	7	21	21
4.00–4.49				4	4				7	7	22	22	11	15		7	19
4.50–4.99						10	5	5	0	10	10	29	24	19	19	24	33
5.00–5.49											8				17	17	17
5.50–5.99											10	10				30	
6.00–6.49													10	10		30	10
6.50–6.99															6	11	6
7.00–7.49														20			10
7.50–7.99																	
8.00–8.49																	
8.50–8.99															8	8	8
9.00–9.49																	
9.50–9.99																	

creased as the fish grew; no erythrophores were observed in specimens >20 mm BL.

Head spination

Major head spines first appeared in specimens at 4–7 mm BL. The posterior preopercular angle spine and anterior preopercular spine appeared at 4.23 mm BL (Fig. 2D), and the posttemporal spine at 6.80 mm BL. The largest specimen lacking anterior and posterior preopercular spines was 4.48 mm BL, and the largest specimen lacking posttemporal spines, 7.21 mm BL (Fig. 2E). No other spines or spinelets of the head appeared in later developmental stages. The number of anterior and posterior preopercular spines increased 2–3 (nine maximum) and 5–7 (nine maximum), respectively, in the size range of 5–16 mm BL (Fig. 2, F

Table 2

recorded for the myomere on which it occurs. *n* indicates the number of specimens examined at each size range.

Myomere																				
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
29	29	7			7			7	7	14	14	21	21	36	43	14	14	7	14	14
	7	22	7		7	19	15	4	11	19	4	19	19	33	30	26	30	15	19	27
7	11	4	4	4	7	11	7	11	15	4	11	15	19	15	19	22	7	7		21
8	8	17			25	8	17	8	17	8	8	8	17	17	17	25	25		8	12
10					10			10	10	10		30	10	20	10	10				10
	20	10	20		20		30	10	20	10	10	50	40	50	10	10			10	10
22		11		6		6		6	6	6	11	6	22	22	6	11	22	6		18
10					10			10	20		10			20	10	30	70	30	20	10
20	20	20	10			20	10	20	0	10	30	20	20	10	30	40	10	40	10	10
54	38	38	31	23	38	31	23	23	15	31	8	31	15	23	8	15	15	8	38	13
92	77	77	77	69	69	69	69	69	62	54	69	69	69	54	54	62	46	38	23	13
83	67	58	75	58	83	58	58	67	58	75	50	50	33	33	25	58	67	33	17	12
100	80	70	90	70	100	70	70	80	70	90	60	60	40	40	30	70	80	40	20	10

Table 3

is recorded for the myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were

Myomere																	
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
29	14	7	57	7	64	29	14	21	79	50	36	57	50	36	43	36	14
15	15	37	11	26	37	41	41	30	44	44	26	26	48	30	33	52	27
19	33	29	29	24	43	38	38	52	24	57	43	43	19	33	29	57	21
17	8	33	17	17	42	42	17	25	83	50	50	50	33	58	33	25	12
20	30	40	60	10	10	60	60	40	70	60	60	30	20	30	20	20	10
30	10	20	60	30	30	10	30	30	40	50	20	0	10	30	20	0	10
6	11	22	28	39	39	33	56	28	44	28	28	22	50	22	17	39	18
	10	30	50	20	50	60	60	40	60	80	50	40	60	30	30	30	10
10	10	20	30	20	40	40	50	50	50	90	70	60	50	70	40	60	10
		8	15	15	23	54	46	62	54	69	62	62	54	62	46	46	13
8		15	8	23	31	54	46	62	46	85	77	54	77	77	85	31	13
				8	25	33	42	58	33	58	67	67	67	75	50	42	12
				10	40	40	40	60	70	80	70	70	70	70	60	40	10

and G). The number of anterior and posterior preopercular spines decreased at 17 mm BL. At >19.23 mm BL, no anterior preopercular spines were observed, except in one specimen (24.00 mm BL) that had two anterior preopercular spines. Juveniles had three posttemporal spines when these spines were fully developed. The largest specimen with a posttemporal spine was 24.23 mm BL, and the largest specimen examined, 37.78 mm BL, had no spines on its head.

Teeth and squamation

Upper and lower jaw teeth were first observed at 5.35 and 6.32 mm BL, respectively. Palatine teeth appeared at 7.20 mm BL and most specimens >9 mm BL had fully developed palatine teeth. At >18.00 mm BL, specimens did not have these teeth.

Squamation was incomplete in the size range examined in this study. The smallest scaled specimen (27.37 mm BL)

Table 4

Incidence of melanophores on the left lateral side of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is recorded for the the first to eighth myomeres.

Size range (BL; mm)	Myomere														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.50–3.99			7	7	7	14	7	29	14	21	7	29	14	7	14
4.00–4.49		4			11	4				7	11	11	11	11	11
4.50–4.99					5	5	5		5	10	5	10	5	29	10
5.00–5.49		8				8				8		8		17	8
5.50–5.99					20		20	20	20	10		40			10
6.00–6.49									20			10		20	10
6.50–6.99				6	0	6	6	6		28	11			17	22
7.00–7.49									20				20	20	
7.50–7.99										20	10	20			20
8.00–8.49						15	8			15		8	8	8	15
8.50–8.99										8		15	8	8	8
9.00–9.49										8				17	17
9.50–9.99	10											10		10	20

Table 5

Incidence of melanophores on the right lateral side of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is recorded for on the first to nineth myomeres.

Size range (BL; mm)	Myomere													
	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.50–3.99	0	7	7	7	14	7	21	14	21	14	29	14	7	14
4.00–4.49	4			11	4	4			11	4	15	11	11	11
4.50–4.99				5				5	10	5	10	5	19	10
5.00–5.49					17	8			17		8		33	8
5.50–5.99	10			20		10	20	10		10	40			10
6.00–6.49								20	10		10		30	10
6.50–6.99		6	6		6	6	6		28	11			17	22
7.00–7.49								20	10	20		10	10	
7.50–7.99					10			10		10	20	20		10
8.00–8.49									31					15
8.50–8.99					15	15					31	31	31	15
9.00–9.49					8				8				17	17
9.50–9.99											10	10		30

had anterior lateral line scales. Scales near the anterior part of the lateral line appeared at 30.31 mm BL, and postorbital scales at 30.81 mm BL.

Discussion

Development and growth

Thunnus thynnus eggs were reported to have a smooth chorion, narrow perivitelline space, homogeneous yolk, and a single oil globule (Miyashita et al., 2000), and this was reconfirmed in our study. These characteristics are similar to those found in other *Thunnus* eggs: *T. thynnus* from the Mediterranean (Podoa, 1956), *T. obesus* (Kikawa, 1953), *T. albacares* (Harada et al., 1971b; Mori et al., 1971), and *T. alalunga* (Yoshida and Otsu, 1963).

The growth strategy of bluefin tuna apparently is to develop foraging structures before other organs and at an early stage to enable feeding on larger organisms. Parts of the head were large from early development (Fig. 3) as mentioned by Collette et al. (1984) and reached maximum

Table 4

myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed on

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
14	36	7		21	7	7	14	14	7	14	7		14	7		14
	30	11	7	15	4	4	11		11	4	7	11	7	7	7	27
14	10	19	14	5	5		5	5	19	10	14	5	10		5	21
17	8	8	8	33				8	17	25	8	8				12
20		10	10	20		20	40		10	10	30	10				10
	10	10		20	10	20			10							10
11	11	6	11	17	6	6	11	6	11	6	17	11		6		18
		20	10	10			10		10	20	10	10	10		10	10
		20	20	10	20	20		30		10	10	40		20	10	10
8		8	15	8		15	31	31		8		8		8		13
15	8	8	31	15	23	15		23	15	15	8	8	8	8	8	13
8	8	17	17	25	25	42	58	33	50	33	50	33	33	50	42	12
	10	10		20	20	20		50	60	40	50	40	30	20	50	10

Table 5

the myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
14	36	7		21	7	7	14	14	14	7	7		14	7	7	14
4	30	19	7	15	7	4			15	4	7	15	19	7	7	27
14		10	14		5			5	19	5	5				5	21
17	25	17	17	42			25	8	17	8	17	8				12
10			30	20		20	10		10	50	30	10				10
10	10	10		10	20	10		10	10	10						10
6	11	11	11	17	6	6	11	6	11	6	17	11		6		18
		10	10	30	10	10	10	10			20	20	20			10
	10	10	30	20	10			10		10	20	30		30	20	10
31		8	8	8		15	15			8		8		8		13
8	15	15	54	23	31	46	46	31	31	31	8		8	23	15	13
8	17	8	8	25	25	42	50	50	42	25	42	25	25	42	25	12
	30	20		20	20	10	10	30	30	40	50	50	30	20	50	10

ratios in relation to BL at sizes smaller than other body parts, such as total length and body height, except for the caudal peduncle depth (Fig. 4).

Another ontogenetic characteristic of growth in *T. thynnus*, the posterior migration of the anus (Collette et al., 1984), was also confirmed: the anus initially positioned in the anterior part of the body, was located at the center of the body at late flexion, and thereafter in the posterior part of the body (Fig. 3).

Kaji et al. (1996) examined the relative growth of larvae of the Pacific bluefin tuna at 3–14 mm BL and reported that body proportions showed constant relative growth values from 10 mm BL, except for preanal length. Our results showed that at 10 mm BL, constant ratios had not been reached in relation to BL. This difference may be due to an insufficient number of larger specimens in Kaji et al.'s (1996) study. Even at the juvenile stage, ratios in relative BL decreased gradually, except for a few cases (Fig. 3), suggesting that body proportions of juvenile bluefin tuna are different from those of adult fish.

Early growth is rapid in *T. thynnus* compared with other marine fishes cultured in Japan (e.g. red sea bream,

Table 6

Incidence of melanophores embedded in the lateral muscle and on the myosepta (%) in *Thunnus thynnus*. Each melanophore is recorded on the first to nineth myomeres.

Size range (BL; mm)	Myomere 10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.50–3.99														
4.00–4.49										4				
4.50–4.99	4	4	4	7	4			4	4	4	4		7	
5.00–5.49						8			8	8				
5.50–5.99			10								10			
6.00–6.49				10		10	10	10	10					
6.50–6.99														
7.00–7.49	10	10		10			10	20	10	10		10		
7.50–7.99			10			10				10		10		
8.00–8.49			8	8	8	8	8	8	8	8	15	23	31	15
8.50–8.99			8	0	8	8	0	0	15	31	38	46	54	62
9.00–9.49			8	8	8	8	25	33	33	42	50	50	50	58
9.50–9.99			10	10	10	10	30	40	40	50	60	60	60	70

Table 7

Incidence of melanophores on the dorsal fin base (%) in *Thunnus thynnus*. Each melanophore is recorded for the nearest myomere. for larvae <7.50 mm BL.

Size range (BL; mm)	Myomere 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
7.50–7.99	10	10	10	10	10	20	20	20	30	20	30	20	30	20	20	20	20	10
8.00–8.49	8	8	8	8	8	15	15	15	23	15	23	15	23	15	15	15	15	8
8.50–8.99		8	15	31	46	46	46	46	46	54	38	54	31	31	31	31	46	46
9.00–9.49	8	17	25	50	58	83	83	83	83	83	83	83	83	83	83	83	83	83
9.50–9.99		20	40	70	80	100	60	60	60	60	100	100	100	100	100	90	100	100

Table 8

Incidence of melanophores on the anal fin base (%) in *Thunnus thynnus*. Each melanophore is recorded for the nearest myomere. *n* indicates the number of specimens examined at each size range. Melanophores were not observed on the first to twenty-first myomeres and for larvae <7.0 mm BL.

Size range (BL; mm)	Myomere 22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
7.00–7.49			10	10	20	10	20	20	20	10	10	10	10	10					10
7.50–7.99	10	20	30	30	50	40	60	40	40	40	40	20	30	20	20	20	10		10
8.00–8.49		15	8	15	31	38	31	62	69	54	62	62	46	31		15			13
8.50–8.99		8	8	8	8	31	31	38	62	69	69	69	69	69	62	62	38	23	13
9.00–9.49					17	33	42	58	75	83	83	75	67	67	50	42	33	25	12
9.50–9.99					20	40	50	70	90	100	100	90	80	80	60	50	40	30	10

Table 6

for the myomere in which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
																14
																27
																21
8																12
																10
		10									10					10
		6				6							11	11		18
10	10	10				10	10						10			10
	10	10	10	10	30	20	30	20		10	30	20	20	20	30	10
23	23	31	23	38	31	54	38	23	38	15	38	38	31	15	15	13
69	62	69	77	77	77	77	77	77	69	62	62	62	62	46	23	13
75	75	83	83	75	83	75	75	75	75	75	75	58	58	67	33	12
90	90	100	100	90	100	90	90	90	90	90	90	70	70	80	40	10

Table 7

n indicates the number of specimens examined at each size range. Melanophore was not observed on the first to fourth myomeres and

Myomere																	
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
10	10	20	20	30	20	30	20	20	30	20	10	10					10
8	8	15	15	23	15	23	15	15	23	15	8	8					13
31	46	38	46	46	38	46	46	38	38	31	46	23		8	0	15	13
83	83	83	83	83	83	83	83	83	83	58	58	58	42	42	8		12
90	90	100	100	100	100	80	80	90	80	80	70	60	20	30	10	20	10

Pagrus major, and white trevally, *Pseudocaranx dentex* [Sawada unpubl. data; Fig. 5]). Rapid growth in bluefin tuna is pronounced after day 20 ($\bar{x}$=9.34 mm BL on day 20) when larvae begin to metamorphose into juveniles. This type of growth phase seems common to scombrids, for example, *T. albacares* (Harada et al, 1971b; Kaji et al., 1999), *Scomber japonicus* (Watanabe, 1970), *Auxis tapeinosoma* (=*A. rocheri*, Harada et al., 1973), and *Sarda orientalis* (Harada et al., 1974) and reaches its height by the time of development of external physical features and the digestive system (Kohno et al., 1984; Miyashita et al., 1998), and by the time of development of their selective feeding characteristics (Sawada et al., 2000).

On day 19 ($\bar{x}$=10.17 mm TL), the mouth size of *T. thynnus* averaged 2.54 mm according to Shirota's (1970) calculation (mouth size index=upper jaw length × $2^{0.5}$). This size is comparable to that of other scombrids, such as *T. albacares* (3.3 mm) and *Katsuwonus pelamis* (3.0 mm) at 10 mm TL, although much larger than that of other species (see Table 2 of Shirota, 1970). The digestive system of *T. thynnus* develops earlier than that of other fishes (Richards and Dove, 1971; Miyashita et al., 1998) and attains an adultlike structure by the juvenile stage, thus allowing bluefin tuna to use food efficiently early in development.

The development of pigment, spines, jaw teeth, and squamation in *T. thynnus* is summarized in Figure 6. The jaw teeth of *T. thynnus* first appeared at 5.35 mm BL (day 10), the palatine teeth at 7 mm BL (flexion stage), and were fully developed at 9 mm BL (postflexion stage). At 7 mm BL, *T. thynnus* larvae fed on other fish larvae and at 8 mm BL (postflexion stage), they were cannibalistic. Teeth appearance and development corresponded to the piscivorous stage during the fast-growth phase after the postflexion stage. Future improved laboratory-rearing techniques

Table 9

Number of erythrophores on the body of *Thunnus thynnus* larvae and juveniles.

Body length (mm)	Dorsal edge	Ventral edge	Lateral midline	Lower jaw	Hypural plate	Caudal fin finfold
3.38						
3.41						
4.63	2	34	5			
4.75	8	32	4			1
5.02	4	2				
5.09	4	36				
5.11	3	54	4			
5.35		24	4			
5.78	1	2	4			
6.10	1	24	5	4		
6.72	3	1	6			
6.91	1	1	4			
6.96	2	17	6	2	2	
6.96		1	8	3		
7.03	8		6		1	
7.06	8	12	8	1	5	2
7.12		8	10		5	
7.20	6	8	6	1	3	1
7.92	1	8	6	1		
8.16		6	8	3		
8.41	1	11	8	3		
8.74	1	4	5			
8.81	4	7	34	2		
8.92		3	3	1		
9.02				1		
9.35	3	12	5	3		
9.38		3	2	3		
9.56		2	2	1		
9.60		9	3	1		
9.70	5	1	6			
9.93	2	2	2			
10.03		1	23			
10.56	4	1	2	7		
10.97	4	1	3			
11.26	1	15	12	2		
11.71						
12.20			4			
13.71		5				
14.00						
14.79		3	1			
14.83		1				
15.33	2	4	1			
15.49						
15.85	1	3	2			
16.86						
18.73		1				
19.71						
19.72		2				
20.40						
21.87						
24.85						
25.89						

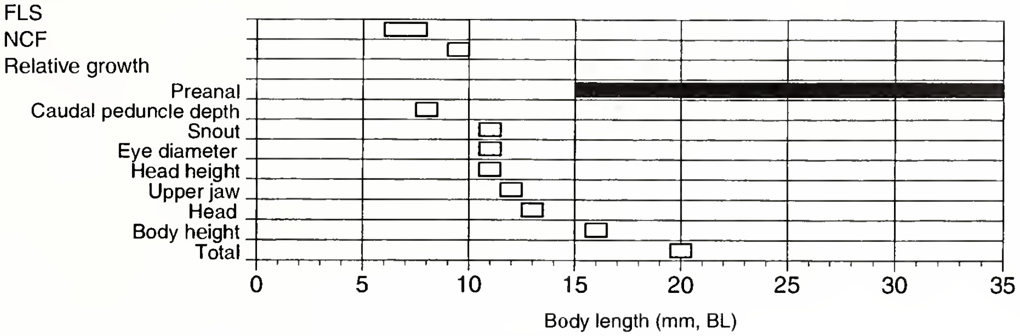

Figure 4

Schematic representation of the relative growth (length of body parts in relation to body length) in hatchery-reared bluefin tuna, *Thunnus thynnus*. Flexion larva subdivision (FLS) and numerical complement of fin rays (NCF) are also shown. In relative growth, ☐ = the peak value of body part proportion (in relation to BL) and ■ = the attainment of a constant value of body part proportion (in relation to BL).

will allow further study of the structure and development of feeding-related bony elements for *T. thynnus*, as has been the case for *S. japonicus* (Kohno et al., 1984) and *Lates calcarifer* (Kohno et al., 1996).

Pigmentation

Accurate identification of *Thunnus* larvae requires an extensive knowledge of individual, growth-associated, and geographic variations in melanophore and erythrophore patterns. We obtained information on the individual and growth-associated variations of these patterns from laboratory-reared specimens.

The melanophore distribution pattern of *T. thynnus* larvae and juveniles showed four distinct characteristic periods of development: newly hatched, 1–3 days after hatching, preflexion to postflexion, and juvenile.

T. thynnus thynnus larvae <3.99 mm BL from the Mediterranean had embedded melanophores (Kohno et al., 1982); but we did not observe these in our specimens until 4.49 mm BL. This difference may be a subspecific difference between *T. thynnus orientalis* and *T. thynnus thynnus*, or it may be due to insufficient numbers of specimens larger than 6.0 mm BL in Kohno et al.'s study, or to the experimental culture product as mentioned later.

Wild-caught *T. thynnus orientalis* larvae (3.15–8.20 mm BL) most frequently had two melanophores both on the dorsal and ventral edges of the trunk and tail (Nishikawa, 1985). This pattern resembles that of *T. thynnus thynnus* larvae from the Mediterranean (2.53–5.25 mm BL, Kohno et al., 1982). But our cultured specimens in the same size range had greater numbers of these melanophores (Tables 2 and 3). Nishikawa (1985) reported the appearance of melanophores at the dorsal fin somewhat earlier than we observed their appearance, but their appearance on the lower and upper jaws occurred in the same size range in both studies where specimens were examined from the same population. Laboratory-reared fish larvae have been shown to have greater pigmentation than wild-caught larvae (e.g. *Pagrus major* [Fukuhara and Kuniyuki, 1978], *Sparus sarba* [Kinoshita, 1986], *Parapristipoma trilineatum* [Kimura and Aritaki, 1985], and *Nibea mitsukurii* [Kinoshita and Fujita, 1988]). Thus, the source of specimens, wild-caught or laboratory-reared, may account for observed differences in larval pigmentation between our study and Nishikawa's study (1985).

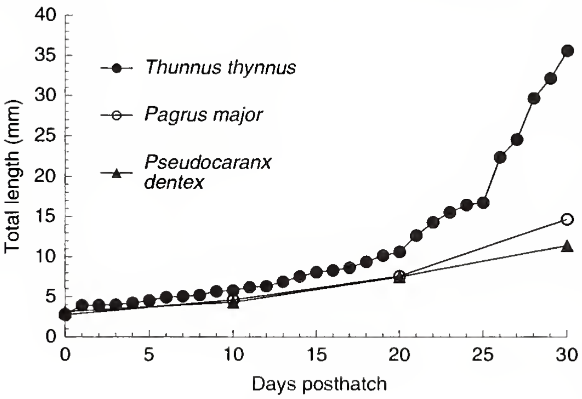

Figure 5

Growth of bluefin tuna (*Thunnus thynnus*), red sea bream (*Pagrus major*), and white trevally (*Pseudocaranx dentex*). Red sea bream and white trevally were reared at the same temperature range (24–27°C) as bluefin tuna (Sawada, unpubl. data).

A slight difference in the occurrence of lower jaw, trunk, and tail melanophores has been reported among *T. thynnus* from the Mediterranean (Kohno et al., 1982), the Atlantic (Richards and Potthoff, 1974), and the Pacific (Matsumoto et al., 1972). Lower jaw melanophores appeared in all specimens larger than 3.0 mm BL from the Atlantic

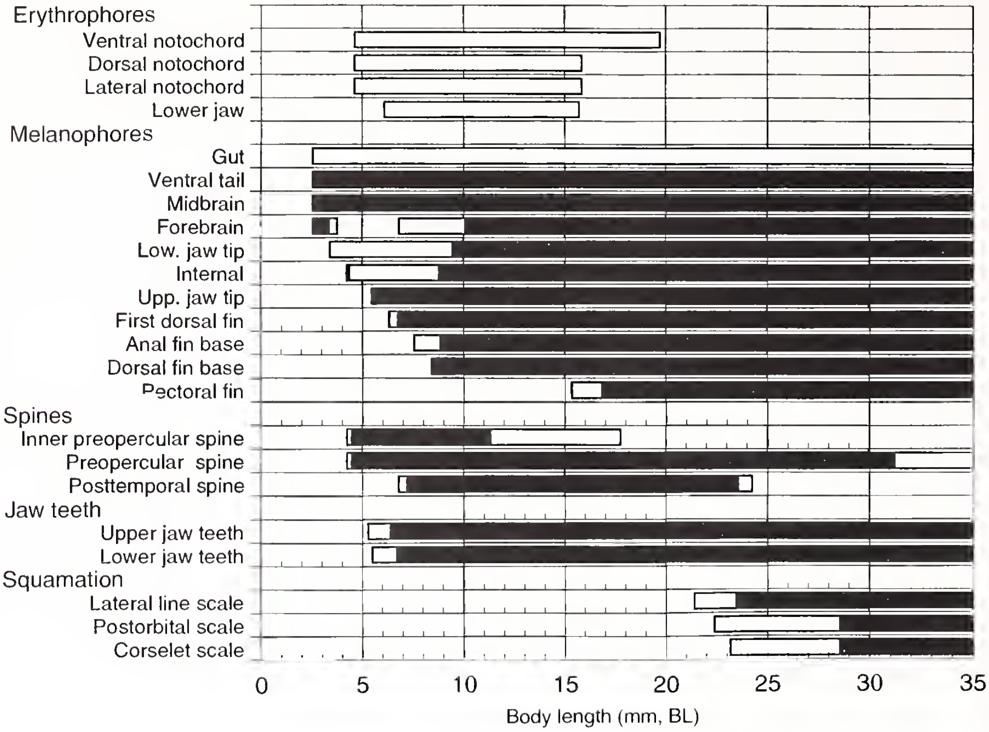

Figure 6

Schematic representation of the development of pigment, spines, jaw teeth, and squamation in hatchery-reared bluefin tuna (*Thunnus thynnus*). ☐ = appearance of pigment and spines was subject to individual variation; ■ = pigment and spines were present in all specimens examined.

and larger than 4.0 mm BL from the Pacific. In our specimens, the incidence of these melanophores was 7.1% at 3.50–3.99 mm BL, 29.6% at 4.00–4.49 mm BL, 57.1% at 4.50—4.99 mm BL, 83.3% at 5.00–5.49 mm BL, and more than 90% in specimens >5.50 mm BL. The largest specimen we observed lacking these melanophores was 9.46 mm BL. Our data are similar to those for specimens from the Mediterranean. The difference in lower jaw melanophore distribution between our specimens and those of Matsumoto et al. (1972) might be explained by geographic variation of the melanophore pattern in the Pacific bluefin tuna. Further study is needed to confirm this hypothesis.

Ueyanagi (1966) reported species-specific characteristics of the erythrophore distribution pattern of Thunninae larvae from the Pacific Ocean: *T. alalunga* consistently had more erythrophores on the dorsal edge of the trunk and tail in front of the caudal peduncle than did *T. albacares*, which had only one or two erythrophores at the caudal peduncle; *T. thynnus* and *T. obesus* had patterns transitional between those of *T. alalunga* and *T. albacares*. Our data for *T. thynnus* generally agreed with those of Ueyanagi (1966); however, the number of dorsal erythrophores in one individual ranged more (0–8) than in Ueyanagi's study (1–5). In addition, ventral erythrophores appeared in large numbers (more than 20) at the preflexion stage (4.63–6.10 mm BL). From examinations of tuna larvae taken in Hawaiian waters, Matsumoto et al. (1972) considered the erythrophore pattern useful as a morphological characteristic for identifying tuna larvae. At present, however, information is limited on erythrophore patterns of other Thunninae larvae and juveniles and on the difference of these patterns in wild-caught and laboratory-reared specimens. Thus collection of such information is needed to establish the erythrophore pattern as a species-identifying characteristic of Thunninae larvae and juveniles.

Thunnus thynnus did not have xanthophores from hatching to the preflexion stage. However, *T. albacares* (Harada et al., 1971b; Mori et al., 1971) and *T. obesus* (Yasutake et al., 1973) have clusters of xanthophores in the finfolds of both the dorsal and ventral fins, and on the dorsal body, respectively. Thus, the xanthophore pattern can be used for distinguishing these *Thunnus* species at the preflexion stage.

Much additional work on development of tunas under controlled conditions, as well as the study on wild-caught materials, is needed to understand their early life history. We believe recent progress in the technology of rearing tunas will yield important information on the early life history of *Thunnus* spp.

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Age and growth of red snapper, *Lutjanus campechanus*, from an artificial reef area off Alabama in the northern Gulf of Mexico

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Abstract—Age and growth were examined of red snapper, *Lutjanus campechanus*, captured in an extensive (3100 km^2) artificial reef area off Alabama in the northern Gulf of Mexico. Sagittal otoliths were removed from individuals (n=1755) sampled from recreational catches and tournament landings. Marginal increment analysis of sectioned otoliths revealed that a single opaque zone formed annually in sagittae from January through May. Fish ages were estimated from the number of opaque zones in otoliths, timing of opaque zone formation, sampling date, and a presumed birthdate of 1 July. Estimated growth of recaptured red snapper (n=288) from a tagging experiment was similar to growth estimated from otolith-aged fish and corroborated otolith aging methods. The von Bertalanffy growth function fitted to length-at-age data was $TL = 969 \text{ mm } (1-e^{-0.192(t-0.020)})$ (P<0.001; r^2=0.99), which was similar to reported growth functions for western Gulf of Mexico and Atlantic red snapper. Results of our study are consistent with the single stock hypothesis for Gulf of Mexico red snapper.

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Red snapper, *Lutjanus campechanus*, are large, predatory reef fish belonging to the family Lutjanidae. They generally are found from Cape Hatteras, North Carolina, to the Yucatan Peninsula, including the waters of the Gulf of Mexico (GOM) but not the Caribbean Sea (Hoese and Moore, 1998). Throughout their range red snapper are distributed along the continental shelf out to the shelf's edge and demonstrate affinity for vertical structure. Adults aggregate on or near coral reefs, gravel bottoms, or rock outcrops, as well as on artificial reefs, oil rigs, and wrecks (Moseley, 1966; Szedlmayer and Shipp, 1994; Stanley and Wilson, 1997).

Red snapper support economically valuable recreational and commercial fisheries in U.S. waters of the GOM (GMFMC, 1989). Federal management of GOM red snapper is based on the assumption that fish from Florida to Texas constitute a single stock. Although genetic evidence supports this assumption (Camper et al., 1993; Gold et al., 1997; Heist and Gold, 2000), fish are not distributed uniformly across the northern GOM. Fisheries-dependent data suggest there is a center of red snapper abundance off southwest Louisiana and a second, smaller center of abundance off Alabama (Goodyear, 1995a; Schirripa and Legault, 1999). For example, from 1981 to 1998 estimated Louisiana landings of red snapper (commercial and recreational catch from state and federal waters) accounted for 32.6% (mean) ±1.5% (SE) of the total GOM harvest, whereas Alabama landings accounted for 11.4% ±0.9% of the total catch (Schirripa and Legault, 1999).

Although fewer red snapper are harvested from waters off Alabama than from the northwestern GOM, the red snapper fishery off Alabama is unique in several ways. Given that Alabama's GOM coastline represents only about 3.0% of the coastline from Tampa, Florida, to Brownsville, Texas, a disproportionately high percentage of the GOM red snapper harvest is caught and land-

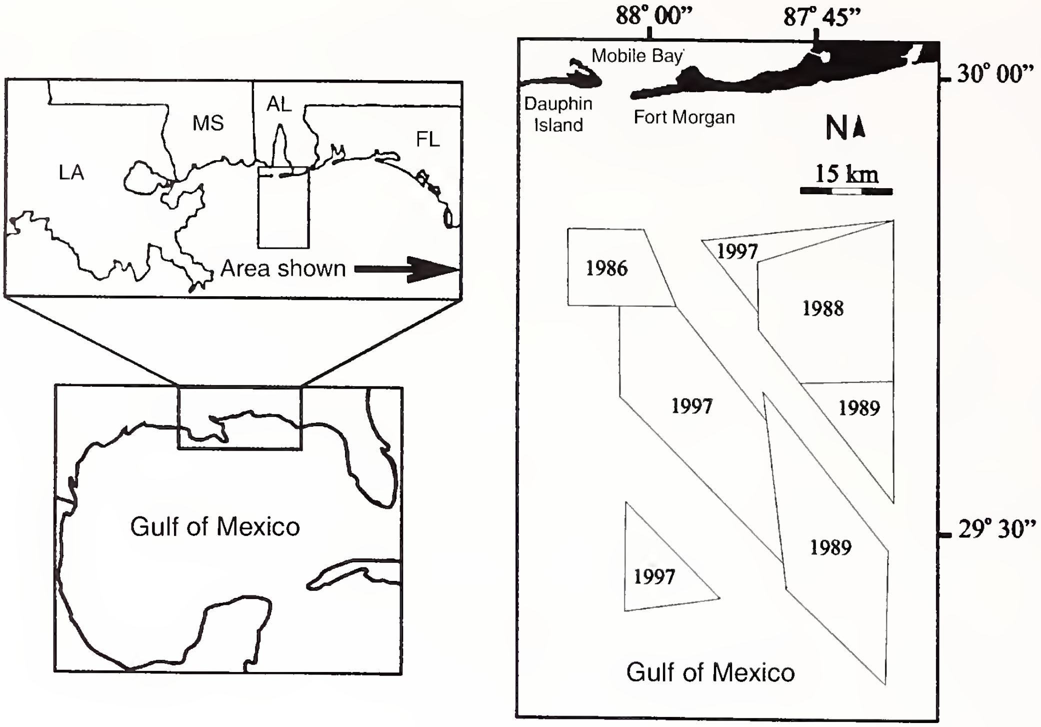

Figure 1

Map of artificial reef permit areas off Alabama. Date of creation is given for each area.

ed there. The productivity of the red snapper fishery off Alabama occurs despite the fact that few high-relief (>1 m) natural reefs exist on the continental shelf in the north central GOM (Parker et al., 1983; Shultz et al., 1987; Schroeder et al., 1989); however, off the coast of Alabama exists a 3100-km^2 area designated for artificial reef deployment (Fig. 1). The correlation between high catch rates and the creation of artificial reefs off Alabama has caused some to speculate that artificial reefs have increased the productivity of the GOM red snapper stock in this area (Szedlmayer and Shipp, 1994; Minton and Heath, 1998).

Despite the implied effect of artificial reefs on the red snapper fishery off Alabama, few studies have focused on red snapper in this area. The objective of our study was to estimate growth rates of adult red snapper captured off Alabama with otolith-aging and mark-recapture methods and to compare growth estimates with growth of adult red snapper from the western GOM and the southeastern United States. As a corollary to our primary objective, we also attempted to validate presumed annual growth rings in otoliths with marginal increment analysis and present a comparison of estimated growth of otolith-aged red snapper with estimated growth of tagged individuals.

Methods

Otolith aging

Red snapper were sampled from July 1995 to September 1999. All fish were caught over artificial reef sites off Alabama. Red snapper shorter than the legal size limit (380 mm total length [TL] for most of the study) were randomly sampled from undersize fish caught during research cruises to tag red snapper. Fish longer than the legal size limit were either randomly sampled from recreational catches or sampled opportunistically at spearfishing or hook-and-line fishing tournaments. Total length and fork length (FL) were measured to the nearest mm for all fish, and whole weight was measured to the nearest 0.1 g. Sex was determined for most fish by macroscopic examination of the gonads. Both sagittae were removed from each sampled individual, rinsed of any adhering tissue, and stored in paper coin envelopes until processing.

Otoliths were sectioned in a transverse plane following the methods of Cowan et al. (1995) and were read under transmitted light with either a Micro Design® model 925 microfiche projector or an Optimas® image analysis system (Media Cybernetics, 1999). Otoliths were read independently by two readers. Blind counts of opaque zones of each sectioned otolith were made along the ventral margin of the sulcus acousticus from the core to the proximal surface; marginal increments were scored following Beckman et al. (1991) (Table 1, Fig. 2). Otoliths for which counts of opaque zones differed between readers were read a second time. Precision among readers was evaluated with the coefficient of variation (CV) (Chang, 1982), index of precision (D) (Chang, 1982), and average percent error (APE) (Beamish and Fournier, 1981).

Age was estimated from the number of opaque zones in otolith sections, timing of opaque zone formation, assumed birthdate, and sampling date. It was assumed that opaque

Table 1

Scale used in determining condition of red snapper otolith marginal increments.

Margin score	Margin description
1	Opaque zone begins to form at edge; zone is <1/3 the thickness of the previous opaque zone.
2	Opaque zone at edge is between 1/3 and 2/3 the thickness of the previous opaque zone.
3	Opaque zone at edge is >2/3 the thickness of the previous opaque zone.
4	Translucent zone begins to form at edge; zone is <1/3 the thickness of the previous translucent zone.
5	Translucent zone at edge is between 1/3 and 2/3 the thickness of the previous translucent zone.
6	Translucent zone at edge is >2/3 the thickness of the previous translucent zone.

zones constitute annuli and that annulus formation begins 1 January (see "Results" section). The birthdate for red snapper in the north central GOM was assumed to be 1 July, which follows the convention of Goodyear (1995a) and is based on the peak in red snapper spawning in the north central and northeastern GOM (Collins et al., 1996; Szedlmayer and Conti, 1999). According to these assumptions, young-of-the-year northern GOM red snapper form their first annulus in sagittae beginning in January when fish are approximately 0.5 yr old. Therefore, the opaque zone closest to the otolith core represents only 0.5 yr of life, which was accounted for in the aging algorithm.

Age (in d) was estimated (for most fish) by first subtracting one opaque zone from the total number of opaque zones in a given otolith and multiplying the difference by 365 d. Next, 182 d was added to the product to account for the first 0.5 yr of life. Finally, the day of year (number of days since 1 January) the fish was sampled was added to account for the number of days in the sampling year that the fish was alive. The result was divided by 365 d to estimate age in years. Age was estimated similarly for fish that were sampled in November and December and had already begun forming opaque margins, except two was subtracted from the total number of opaque zones before multiplying by 365 d in order to assign fish to the correct year class. For fish that were sampled in January that did not have an opaque margin, zero was subtracted from the total number of opaque zones.

Von Bertalanffy (1938, 1957) growth functions (VBGFs) were fitted to TL-at-age data with Proc NLIN in SAS (SAS Institute, Inc., 1996). Von Bertalanffy growth functions were fitted separately for males and females and a likelihood ratio test was used to test for difference in growth between sexes (Kimura, 1980; Cerrato, 1990). Von Bertalanffy growth functions also were fitted for the complete data set and for the complete set excluding tournament sampled fish.

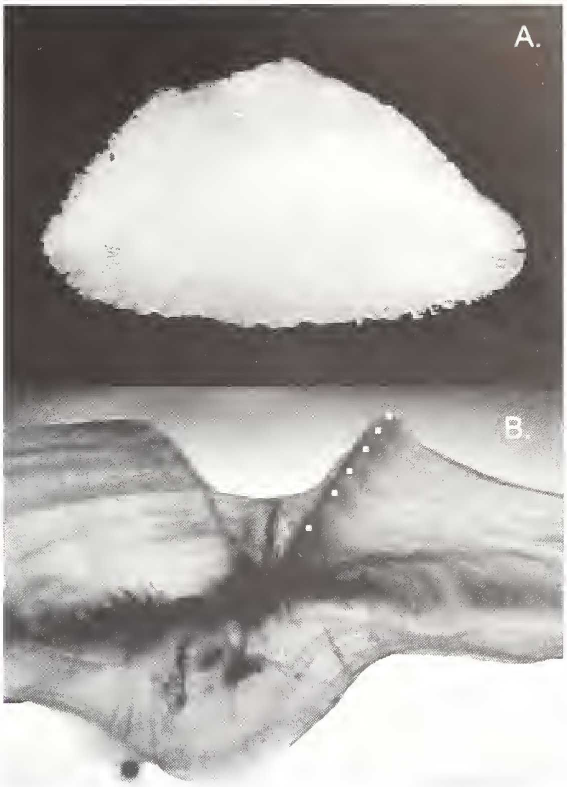

Figure 2

Digital images of (**A**) the proximal view of the left sagitta from a 408-mm-TL female red snapper sampled in August 1996 and (**B**) a thin section of a sagitta from a 683-mm-TL female red snapper sampled in January 1996. White squares on the thin section designate opaque zones (n=6); edge score is one.

Weight-TL relationships were modeled with non-linear regression for females and males following Ricker (1975). The functions were computed with Proc NLIN in SAS (SAS Institute, Inc., 1996). Sex-specific weight-TL relationships were made linear by taking the log of weight and TL, and difference between sexes was tested with an analysis of covariance (ANCOVA) on the log-transformed data (SAS Institute, Inc., 1996). Lastly, a weight-TL nonlinear regression was computed for females and males combined with Proc NLIN in SAS (SAS Institute, Inc., 1996).

Mark-Recapture

A tagging study of adult red snapper was conducted off Alabama from March 1995 to August 1999. Fish were caught with hook and line over nine artificial reef tagging sites in the Hugh Swingle general permit area for arti-

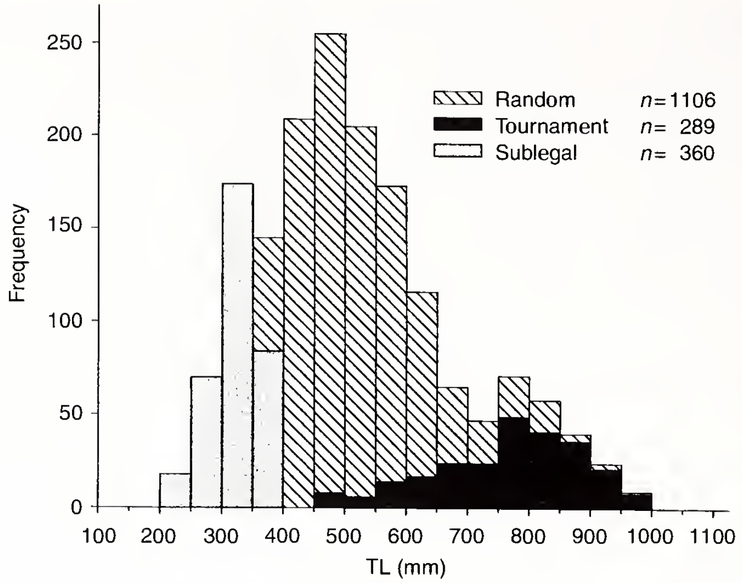

Figure 3

Distribution of total length (TL) of red snapper sampled for age and growth estimation.

ficial reef deployment (labeled 1986 in Fig. 1). Each fish was measured to TL and FL, tagged with a yellow Floy® (Seattle, WA) internal anchor tag, and released. Individuals were recaptured on subsequent tagging trips or were recovered by recreational and commercial fishermen. Red snapper recaptured on tagging trips were measured to TL and FL and recovered fish were measured when fishermen retained carcasses of tagged fish.

To corroborate red snapper age estimates, VBGF parameters estimated from otolith-aged fish were incorporated into Fabens' (1965) length increment model to predict TL at recapture of tagged fish (Labelle et al., 1993; Thompson et al., 1999). Solving for TL at recapture, Fabens' model is computed as

$$TLr_i = Tl_i + (L_\infty - t_i)\,(1 - e^{-k\Delta t_i}), \tag{1}$$

where TLr_i = predicted TL at recapture of individual i;
TL_i = TL at release of individual i;
L_∞ = TL asymptote from VBGF estimated from otolith-aged fish;
K = growth coefficient from VBGF estimated from otolith-aged fish; and
t_i = time at liberty of individual i.

Predicted TL at recapture from Fabens' method was plotted against observed TL at recapture to compare growth model predictions to observed values.

Growth of tagged red snapper was estimated by regressing their change in TL on days at liberty (SAS Institute, Inc., 1996). A linear regression also was computed on TL-at-age data over the size range of recaptured individuals from the tagging study (SAS Institute, Inc., 1996). Slopes of resultant regressions were compared to assess if estimated growth rates were different between tagged and otolith aged fish.

Results

Age and growth

Sagittae were collected from 1755 red snapper, including 360 fish shorter than the minimum size limit (380 mm TL) caught on tagging cruises, 289 fish from tournaments, and 1106 fish randomly sampled from recreational catches (Fig. 3). Sex was not determined for 279 fish, of which 61 individuals were immature. Mean TL (±SE) was 518.3 (±5.04) mm for males and 529.5 (±5.96) mm for females. Total length of immature individuals ranged from 208 to 309 mm.

Of the 1755 otoliths sectioned for age determination, reader 1 and reader 2 agreed on the number of opaque zones for 1610 (91.7%) fish after the first reading; opaque zones in 23 otoliths were deemed not interpretable owing to sample preparation. Count disagreement between readers was the following: one opaque zone for 123 fish, two zones for 18 fish, and three zones for four fish. Otoliths were read a second time if reader counts were not in agreement and second otoliths were sectioned and read of the 23 fish for which otoliths were rejected after the first reading. After the second reading, agreement was reached for 1676 (95.6%) otoliths, including all 23 otoliths that were second sections. Disagreement between readers after the second reading was as follows: one opaque zone for 71 fish, two zones for 7 fish, and three zones for one fish; fish for which agreement was not reached after the second reading were not assigned ages or included in the growth estimation. Precision estimates were 1.25% for APE, 0.90% for CV, and 0.64% for D after the second reading.

A clear pattern exists in the marginal increment scores, demonstrating that one opaque zone is formed annually in winter (Fig. 4). Most otoliths had opaque margins by January and had translucent margins by June, thus, timing of opaque zone formation appears to be from January through May for most fish. Eight fish (of 118) sampled in November (1997) and six fish (of 66) sampled in December (1996) had opaque margins. Four fish (of 47) sampled in January (1997) had translucent margins.

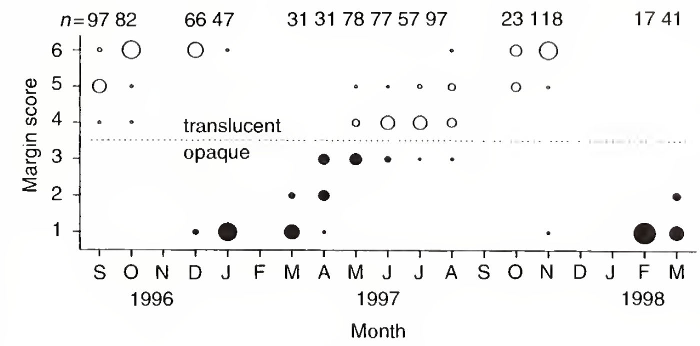

Figure 4

Plot of monthly margin edge scores of red snapper sagittae. Symbol size is relative to the percentage of fish with the corresponding margin edge score. Monthly sample size is given.

The oldest female sampled was 34.1 yr old and the oldest male sampled was 33.2 yr old. The female to male ratio was 1.1:1 overall but was 1.5:1 for fish between 10 and 20 yr old and 3.4:1 for fish greater than 20 yr old (Fig 5, A and B). Von Bertalanffy growth functions for females and males modeled separately were

Females: $TL = 976(1–e^{-0.191(t-0.051)})$
$(F_{3;735} = 21{,}952; P<0.001; r^2 = 0.99)$

Males: $TL = 956(1–e^{-0.194(t+0.054)})$
$(F_{3;726} = 19{,}058; P<0.001; r^2 = 0.99)$.

Computed VBGFs were not significantly different between males and females (likelihood ratio test; $P>0^2_{df=1} = 0.2879$), therefore, sexes were modeled together. Von Bertalanffy growth functions computed for all fish and excluding tournament sampled fish were

All fish: $TL = 969(1–e^{-0.192(t-0.020)})$
$(F_{3;1,672}=47{,}690; P<0.001; r^2=0.99)$ (Fig. 6)

Excluding tournament fish: $TL = 1181(1–e^{-0.120(t+0.652)})$
$(F_{3;1,461}=43{,}550; P<0.001; r^2=0.99)$.

The growth model including all fish was similar to other VBGFs estimated for GOM and southeast U.S. Atlantic red snapper (Fig. 7, Table 2).

Weight-TL nonlinear regression models for females and males modeled separately were

Females: $Weight = (4.46 \times 10^{-9})TL^{3.18}$
$(F_{1;73}= 42{,}577; P<0.001; r^2=0.98)$

Males: $Weight = (5.18 \times 10^{-9})TL^{3.16}$
$(F_{1;728}= 43{,}956; P<0.001; r^2=0.99)$.

Log-transformed weight-TL relationships were not significantly different between males and females (ANCOVA test for equal slopes; $F_{1;1,465}=1.54$, $P=0.2145$; ANCOVA test for equal intercepts; $F_{1;1,465}=1.49$, $P=0.2226$), therefore, sexes were modeled together. The resultant nonlinear regression model was

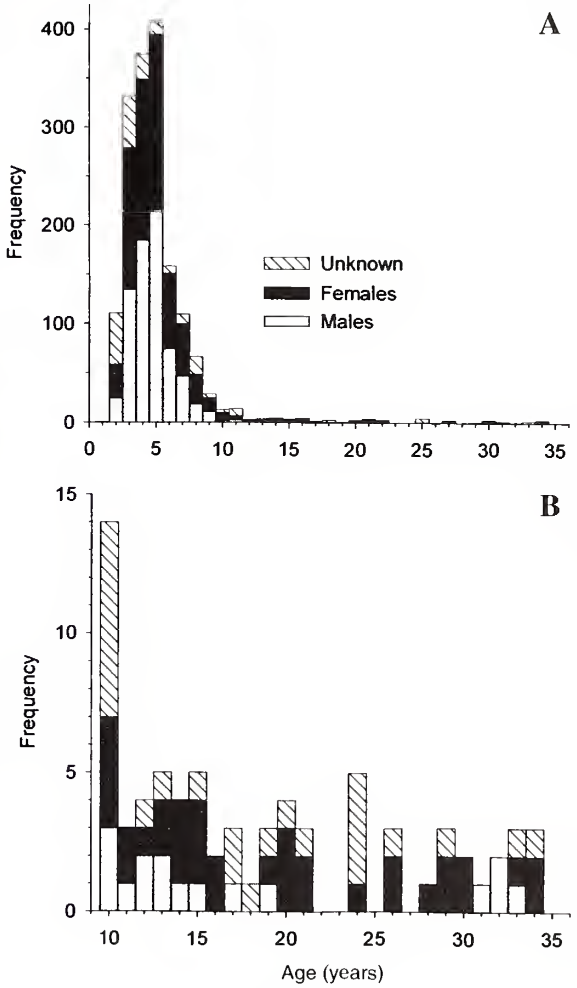

Figure 5

Distributions of age for (**A**) all sampled red snapper and (**B**) all fish greater than 10 yr old. Legend in section A is the same for both distributions.

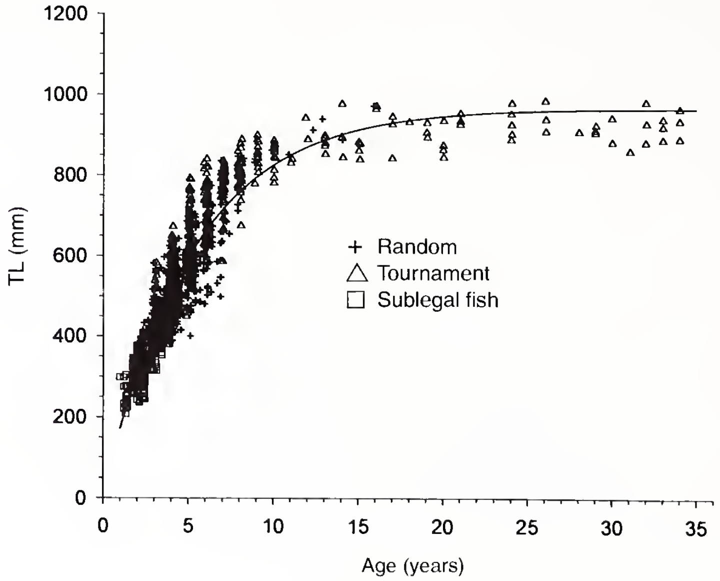

Figure 6

Scatterplot of red snapper TL-at-age data for all fish assigned ages. Plotted line is the von Bertalanffy growth function fitted to the data.

$$Weight = (4.68 \times 10^{-9})TL^{3.17} \quad (F_{1;1,467}=85{,}961;\ P<0.001;\ r^2=0.98).$$

Mark-Recapture

A total of 2932 adult red snapper were tagged. Total length at recapture was measured for 288 of 519 recaptured individuals. Mean TL (±SE) of recaptures was 344 (±4.1) mm (range: 183–660 mm) at release and 423 (±5.6) mm (range: 253–726 mm) at recapture, and mean time at liberty (±SE) was 334 (±16.3) d (range: 12–1501 d). Predicted TL from Faben's method corresponded well to observed TL at recapture for tagged fish (Fig. 8). The linear regression of change in TL on days at liberty was statistically significant ($F_{1;286}$=631.0, $P<0.001$, r^2=0.76) and had a slope of 0.238 mm/d (Fig. 9A). The range in estimated ages of otolith-aged fish for the linear regression of TL on age was 356–2599 d (approximately one to seven years). The model was statistically significant ($F_{1;1,541}$=6309, $P<0.001$, r^2=0.80) and had a slope of 0.240 mm/d (Fig. 9B).

Discussion

Validating the periodicity of opaque zone formation in otoliths as annual is imperative for age and growth studies where otoliths are used as aging structures (Beamish and McFarlane, 1983, 1987). Annual formation of opaque zones in otoliths has been validated for several tropical and sutropical lutjanids (Manooch, 1987; Fowler, 1995; Cappo et al., 2000), and annual formation of opaque zones in red snapper sagittae has been reported from the northwestern GOM and the southeastern U.S. Atlantic (Render, 1995; Manooch and Potts, 1997; Wilson and Nieland, 2001). Our marginal increment analysis of red snapper otoliths demonstrates that opaque zones in adult red snapper sagittae also are formed annually in the north central GOM. The pattern of monthly marginal increment scores reveals that some fish begin opaque zone formation as early as November and some do not have translucent margins until midsummer; however, the general pattern of opaque zone formation is from January through May. Render (1995) and Wilson and Nieland (2001) have reported a similar pattern for adult red snapper in the northwestern GOM, and opaque zone formation in winter–spring has been shown for sagittae of several other teleosts in the northern GOM (Maceina et al., 1987; Beckman et al., 1989, 1990, 1991; Thompson et al., 1999).

Because relatively few old red snapper were sampled in our study, and samples of old fish came mostly from tournaments in summer months, we were unable to validate ages beyond 8 years. Baker (1999) compared age estimates of otolith-aged GOM red snapper with age estimates from radiometric dating of their otolith cores and reported that age estimates from the two methods were highly correlated for fish up to 37 yr old, thus corroborating otolith-based estimates of age for older fish. Therefore, despite lack of annulus validation for older red snapper ages in our study, we are confident that otolith-based age estimates of older individuals are accurate.

Age and growth

Red snapper are long-lived reef fish; we observed a maximum age of 34 yr for females and 33 yr for males. Other

Table 2

Results from previous studies of the age and growth of Gulf of Mexico and southeastern U.S. Atlantic red snapper.

Study and location	Aging structure	Maximum age (yr)	L_∞ mm (TL)	K	t_0
Szedlmayer and Shipp (1994); north central GOM	sectioned otoliths	42	1025	0.150	not reported
Wilson and Nieland, 2001; northwest GOM	sectioned otoliths	53	938	0.175	–0.530
Nelson and Manooch (1982); GOM	scales and sectioned otoliths	15	941	0.170	–0.10
Nelson and Manooch (1982); southeast U.S. Atlantic	scales and sectioned otoliths	15	975	0.160	0.00
Manooch and Potts (1997); southeast U.S. Atlantic	sectioned otoliths	25	955	0.146	0.182

authors have reported fish over 40 yr old (Szedlmayer and Shipp, 1994; Render, 1995; Wilson and Nieland, 2001) and the oldest fish aged to date is 53 yr old (Render, 1995; Wilson and Nieland, 2001). Among western Atlantic lutjanids for which maximum age has been reported, GOM red snapper has the greatest longevity (Acosta and Appledoorn, 1992; Manickchand-Heileman and Phillip, 1996; Potts et al., 1998; Hood and Johnson, 1999). Maximum ages of 30+ and 40+ yr have been reported for several species of Pacific lutjanids (reviewed in Rocha-Olivares, 1998).

Overall, the numbers of sampled males and females were nearly equal, but females were predominant in samples greater than 10 yr old. A similar pattern was observed in Pacific red snapper, *Lutjanus peru*, where the female-to-male ratio was essentially equal (1.1:1) for fish less than 10 yr old and 2.4:1 for fish older than 10 yr (Rocha-Olivares, 1998, Fig. 6). Rocha-Olivares (1998) concluded from catch curve analyses that differences in sex-specific numbers at age for *L. peru* resulted from males experiencing a higher mortality rate. Differential mortality between sexes is not unusual in lutjanids (Grimes, 1987) and the predominance of female GOM red snapper in older age classes may result from a higher mortality rate for males.

Fish sampled from tournaments were included in growth estimation because few large, old individuals were sampled randomly from the recreational catch. The inclusion of tournament sampled fish could bias growth estimates because tournament anglers target large fish; thus the potential exists for them to catch or spear the fastest growing individuals at a given age (Ottera, 1992; Vaughan and Burton, 1993; Goodyear, 1995b). Without the fish sampled from tournaments, however, the VBGF did not reach an asymptote; therefore growth parameters were poorly estimated (Hirschhorn, 1974). We feel that excluding the tournament fish from growth function estimation introduced far greater bias than including them.

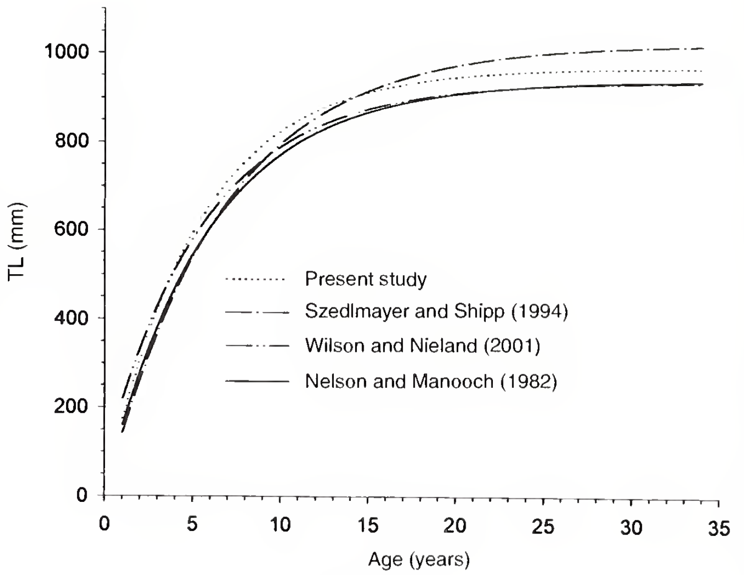

Figure 7

Von Bertalanffy growth functions estimated from TL-at-age data for Gulf of Mexico red snapper. Legend indicates source of each function.

Mark-Recapture

Comparisons between estimated growth of tagged red snapper and otolith-aged fish corroborate otolith aging methods. Predicted TL of tagged individuals obtained with Fabens' (1965) method and VBGF parameters estimated from otolith-aged fish are coincident with observed TL at recapture because predicted and observed values corresponded well to the line of 1:1 agreement. Although ours

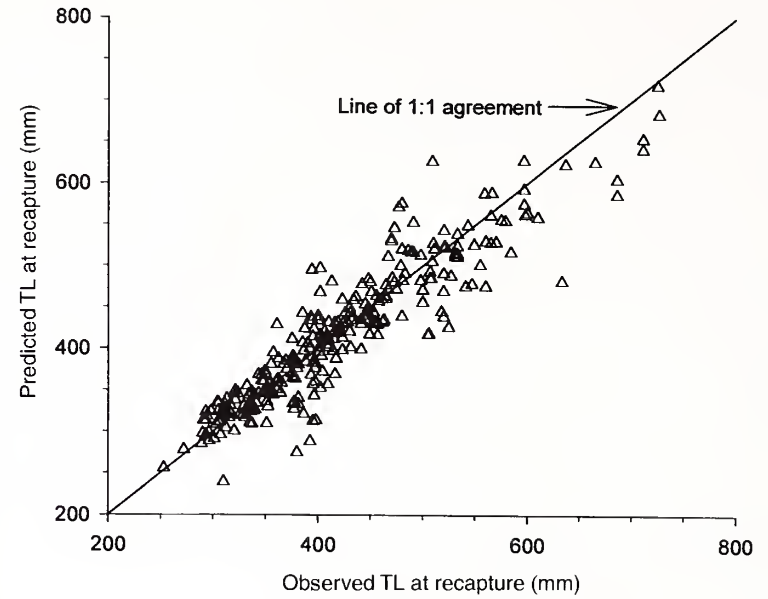

Figure 8

Predicted total length (TL) at recapture computed for tagged red snapper (n=288) plotted versus observed TL at recapture. Diagonal line is the line of one-to-one agreement between predicted and observed values.

was a graphical rather than a statistical exercise (Labelle et al., 1993; Thompson et al., 1999), results corroborate otolith-based estimates of growth.

Direct comparison of linear growth functions computed for tagged fish and otolith-aged fish provides further support for otolith-based estimates of growth (slopes from the two equations differed by only 0.002 mm/d). Francis (1988) cautioned against direct comparison of age-based growth estimates with growth estimates from tagging data because different information results from the different data types. The linear model fitted to length-at-age data predicts TL of fish for a given age (in d), whereas the linear model fitted to tagging data predicts the increment of growth expected of a fish at liberty for a given number of days. However, because the relationship between TL and age is linear over the range of TL from the tagging data (Szedlmayer and Shipp, 1994), we submit that the slopes can be compared as estimates of mean growth. The slope of the age-based linear model is an estimate of the population growth rate of young fish, whereas the slope of the length-based linear model is an estimate of the mean growth of tagged individuals. That growth estimates for otolith-aged and tagged red snapper are very similar corroborates the aging method with otoliths and indicates that on average tagging did not affect fish growth.

Red snapper off Alabama

The red snapper fishery off Alabama is unique in several ways. Despite the reported lack of high-relief natural reef structures on the continental shelf off Alabama (Parker et al., 1983; Shultz et al., 1987; Schroeder et al., 1989), fishermen land a disproportionately high percentage of the annual GOM red snapper harvest from Alabama coastal waters (Schirripa and Legault, 1999). Although Alabama red snapper landings have accounted for approximately 11% of total GOM landings from 1981 to 1998, the recreational fishery off Alabama accounted for mean (±SE) 21.1 (±2.25)% of GOM recreational landings from 1981 to 1998 and 26.5 (±1.83)% of recreational landings from 1990 to 1998 (Schirripa and Legault, 1999). Total landings of red snapper from the U.S. GOM averaged 3.5×10^3 metric tons from 1981 to 1998, with commercial and recreational landings essentially equal; however, 94% of Alabama red snapper landings from 1990 to 1998 came from the recreational sector of the fishery (Schirripa and Legault, 1999).

The red snapper fishery off Alabama is also unique because it is prosecuted almost entirely over artificial reefs (Minton and Heath, 1998; Shipp, 1999). Artificial reef construction began in the 1950s when charter boat operators gained permission from the Marine Resources Division of the Alabama Department of Conservation to place 250 car bodies on the sea floor off Alabama (Hosking and Swingle, 1989; Minton and Health, 1998). Since the 1950s, artificial reefs have been constructed of a variety of materials including car bodies, liberty ships, army tanks, kitchen appliances, newspaper bins, and most recently, prefabricated concrete structures. Reef building increased dramatically in the 1980s with the creation (by permit) of artificial reef areas that now encompass a total of 3100 km^2, and it is estimated that over 15,000 artificial reefs currently exist off Alabama (Hosking and Swingle, 1989; Szedlmayer and Shipp, 1994; Minton and Health, 1998; Havard[1]).

The correlation of catch rates with artificial reef construction has caused some to conclude that artificial reefs off Alabama have increased the production of red snapper, as opposed to merely aggregating fish from surrounding areas (Szedlmayer and Shipp, 1994; Minton and Health, 1998). Among the evidence cited in support of this conclusion was that red snapper grew faster, attained larger sizes, and lived longer over artificial reefs off Alabama than other places throughout their range (Szedlmayer and Shipp, 1994). More recent data, including those from the current study, indicate that GOM red snapper off Alabama grow similarly to and reach similar sizes as fish from the northwestern GOM and the Atlantic (Render, 1995; Manooch and Potts, 1997; Wilson and Nieland, 2001). It is also apparent that the maximum longevity of fish off Alabama is no greater than that of fish of other locations in the GOM (Szedlmayer and Shipp, 1994; Render,1995; Wilson and Nieland, 2001).

These results have important implications for management of GOM red snapper. That growth of fish off Alabama is similar to growth of fish in the northwestern GOM is consistent with the management paradigm that northern GOM red snapper constitute a single stock (Goodyear, 1995a) and contrary to the hypothesis that fish off Alabama are unique (Szedlmayer and Shipp, 1994). Moreover, if fishing mortality rates are higher off Alabama than other places in the northern GOM but growth is the same, production of red snapper may be lower off Alabama than in other areas. Furthermore, if red snapper recruit to artificial reefs off Alabama from other areas in the northern GOM, Alabama's red snapper fishery may serve as a net sink for stock-specific production. Future research is needed to compare mortality as well as growth of fish from different areas in the northern GOM and to estimate the source of recruits to Alabama's artificial reefs.

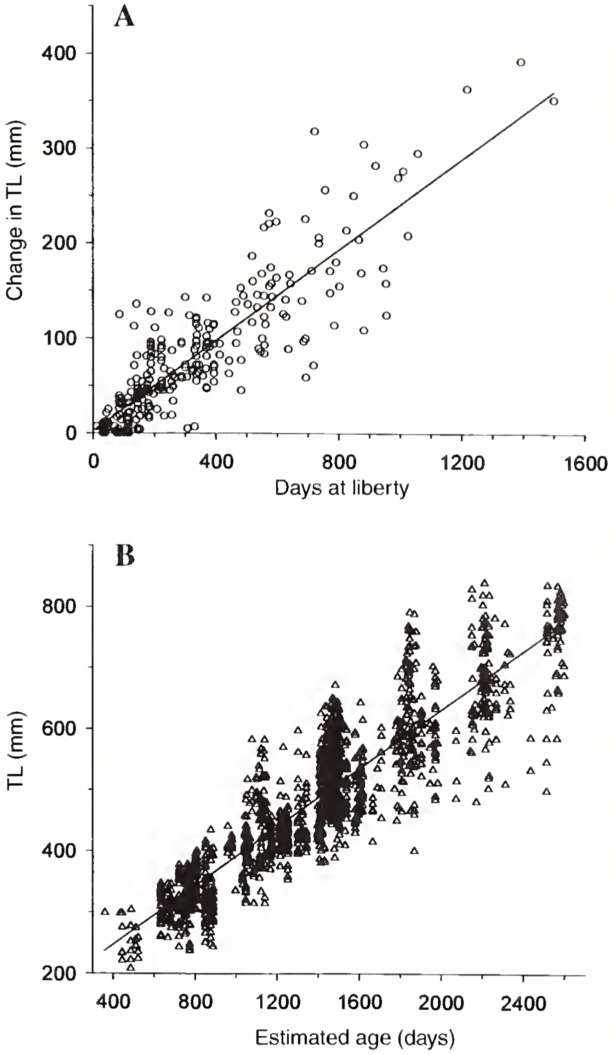

Figure 9

Linear regressions fitted to (**A**) change in total length (TL) versus days at liberty for recaptured red snapper (n=288) from the tagging study and (**B**) TL-at-age data for otolith-aged red snapper in the size range of fish from the tagging study (n=1543).

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A comparison of calcified structures for aging summer flounder, *Paralichthys dentatus**

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Abstract—Calcified structures of summer flounder, *Paralichthys dentatus*, were evaluated to identify the best age determination method. Scales, the currently preferred structure, were compared with opercular bones and to right and left whole and sectioned otoliths for ages 0 to 10. All structures showed concentric rings that were interpreted as annual; however structures differed greatly in the clarity of their presumed annual marks. Right and left otoliths generally gave the same age, although they differed in the clarity of marks. Sectioned otoliths, particularly right ones, were the best aging structure. Right sectioned otoliths consistently showed the clearest marks and had the highest confidence scores, lowest reading times, and highest agreement within and between readers, 97% and 96%, respectively. Left sectioned otoliths took twice as long to prepare and were more difficult to interpret than right sectioned otoliths. Whole otoliths were the second best structure and were adequate to age 4 or 5, after which sectioning greatly improved the clarity of marks. Scales were inferior to, and often did not give the same age readings as, whole and sectioned otoliths. Compared with otoliths, scales tended to overage at younger ages and to underage at older ages. Opercular bones were undesirable for aging summer flounder. They were often unclear and inconsistent, and they had the lowest confidence scores, the highest reading times, and only 46% within-reader agreement. A major source of disagreement in scale and otolith age readings was the presence of an early, presumably false, mark on some structures. We compare the formation of this early mark in summer flounder with early mark formation on otoliths of Atlantic croaker, a species with similar life history traits.

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The summer flounder, *Paralichthys dentatus*, ranges from Nova Scotia to Florida, although it is most abundant from Massachusetts to North Carolina (Ginsburg, 1952; Leim and Scott, 1966; Gutherz, 1967). In regions of high abundance, it is one of the most important commercial and recreational fishes on the Atlantic coast (MAFMC, 1987). In the Chesapeake Bay region, for example, summer flounder support an extensive recreational fishery from about March to November, when they are present in the lower portions of the Chesapeake Bay and in coastal waters (Hildebrand and Schroeder, 1928; MAFMC, 1987; Desfosse, 1995). They then support a strong commercial fishery during the fall and winter, when they move offshore to the continental shelf (Ginsburg, 1952; Bigelow and Schroeder, 1953; Poole, 1962; MAFMC, 1987).

Many studies have reported difficulties with the structures used for age determination of summer flounder. Prior to about 1980, whole left otoliths were the most commonly used structure (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977; Powell, 1982). However, there were disagreements over the location and interpretation of the first presumed annual mark (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977), largely a result of uncertainties about first year growth rates. This and other problems with whole otoliths (summarized in Smith et al., 1981) prompted a comparison of age determination structures by Shepherd (1980), who reported that presumed annual marks were more distinct on scales than on whole otoliths. Consequently, scales became the preferred structure for aging summer flounder (Smith et al., 1981; Dery, 1988; Almeida et al., 1992). More recently, Szedlmayer et al. (1992) examined first year growth rates to resolve the location and interpretation of the first mark on whole otoliths, but scales have remained the preferred structure (Bolz et al., 2000).

Difficulties have also been reported in using summer flounder scales (Dery, 1988; Desfosse, 1995; Bolz et al., 2000). Desfosse (1995) used marginal increment analysis to validate scales for ages 1 to 3. He reported only 46% within-reader agreement past age 4, however, indicating that marks on scales are not very distinct at older ages. He attributed disagreements to false or indistinct annuli and to crowding of annuli at the scale edge in older fish. Most recently, Bolz et al. (2000) reported only 53% agreement for ages 1 to 5 in a between-agency exchange of scales, with agreement increasing to only 83% after they resolved as many disagreements as possible. They attributed most of the remaining disagreements to the choice of a first annual mark and to differing opinions on what constituted a false mark on scales.

A reexamination of calcified structures for aging summer flounder is needed, given their economic importance and the reported difficulties in

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age determination with whole otoliths and scales. Previous studies have never evaluated sectioned otoliths in summer flounder, even though sectioned otoliths have often proven a superior structure in other species, especially at older ages when scales and other structures can underage fish (Beamish and McFarlane, 1983). Further study is especially needed because the location of the first mark on otoliths has recently been determined (Szedlmayer et al., 1992). In addition, no work has been done to determine if right-left differences in the location of the focus result in differences in age determination.

The main objective of our study was therefore to evaluate and compare whole otoliths, sectioned otoliths, scales, and opercular bones for aging summer flounder. We included opercular bones because many studies, on a variety of species, have found them to be superior to other structures and to have very distinct and easy to read marks (for examples, see LeCren, 1947; Donald et al., 1992; Hostetter and Munroe, 1993). A second objective was to compare right and left otoliths for potential differences in age based on differences in the location of the focus. Calcified structures were evaluated in terms of preparation and reading times, confidence in presumed annual mark clarity, agreement between repeated age readings, structure growth with fish growth, age agreement between different structures of the same fish, and increases in the number of presumed annual marks with structure size and fish size. Finally, we discuss the formation of early, presumably false, marks on summer flounder otoliths and scales that resulted in difficulties in age interpretation

Methods

Sample collection

To minimize difficulties interpreting marks on the edge of the structures, collections of summer flounder were made far from the time of presumed annual mark formation, which occurs in May and June on the scales of Chesapeake Bay summer flounder (Desfosse, 1995). Summer flounder were collected from commercial fisheries in the Chesapeake Bay region from September through November of 1998 (n=165). Additional juvenile fish (n=11) were collected by the Virginia Institute of Marine Science juvenile bottom trawl survey in October of 1998 in the lower Chesapeake Bay and James River.

Fish were processed for total length (TL), total weight (TW), and sex, and the calcified structures were removed as follows. Both saggital otoliths were removed, wiped clean, and stored dry in tissue culture cell wells. Scales were removed from just above the lateral line anterior to the caudal peduncle (Shepherd, 1980; Dery, 1988) and stored in coin envelopes. Both opercular bones were removed according to the methods of LeCren (1947), stored in coin envelopes, and frozen.

The collection of summer flounder was stratified into six length-based categories of 100 mm each to include as many age groups as possible in the final study sample. A random sample of 15 fish was then chosen from the first five categories. The last category included the six largest fish, all of which were used in the comparison, for a total of 81 fish. All calcified structures in the final study sample were assigned random numbers before preparation and aging. Summer flounder in the final study sample ranged in size from 209 to 758 mm TL and from 80.8 to 7304.6 g TW and in age from 0 to 10 years (determined from sectioned otoliths, as reported in this study).

Preparation of calcified structures for age determination

Whole otoliths were examined in water on a dark background with reflected light at 120 to 240× magnification. Thin opaque bands, which appeared white under reflected light, were presumed to represent annual marks (Fig. 1A). Two counting paths were used for mark enumeration. The primary counting path was from the focus to the anterior margin of the otolith. The secondary counting path, used to verify the primary counting path reading, was from the focus to the posterior margin of the otolith. With calipers to 0.05 mm, whole otolith total length (WOTL) was measured as the largest distance from the anterior to the posterior edge and whole otolith radial length (WORL) was measured from the center of the focus to the tip of the anterior edge. A paired sample t-test was used to test for right-left differences in WORL.

After all whole otolith readings were made, right and left otoliths were mounted sulcal groove down onto cardboard with crystal bond adhesive and sectioned transversely through the focus with a variable speed Beuhler Isomet saw. The resulting sections, about 0.5 mm thick, were mounted on clear glass slides and immersed in crystal bond. Sections were viewed with transmitted light and bright field at 240× magnification. Thin opaque bands, which appeared dark with transmitted light, were presumed to represent annual marks (Fig. 1B) and were counted along the ventral side of the sulcal groove. Sectioned otolith radial length (SORL) was measured to 0.001 mm along the ventral arm of the sulcal groove from the center of the focus to the otolith edge by using a compound video microscope with the Optimas image analysis system (Media Cybernetics, 1999). Broken otoliths were not measured if they were fractured along the focus. A paired sample t-test was used to test for right-left differences in SORL.

Opercular bones were prepared according to the methods of LeCren (1947). Briefly, they were soaked in cold tap water for several minutes to thaw and to partially loosen surrounding skin, then soaked for 1 minute in simmering water, after which the skin was easily removed with a toothbrush. The opercular bones were then rinsed with cold tap water and air-dried. Opercular bones were examined dry with transmitted light and in water with reflected light on a dark background. Presumed annual marks (Fig. 1C) were defined as sharp transitions from relatively narrow translucent zones to relatively wide opaque zones that were continuous from the anterior to the posterior margin of the bone (Bagenal and Tesch, 1978; Hostetter and Munroe, 1993). Translucent zones appeared white under

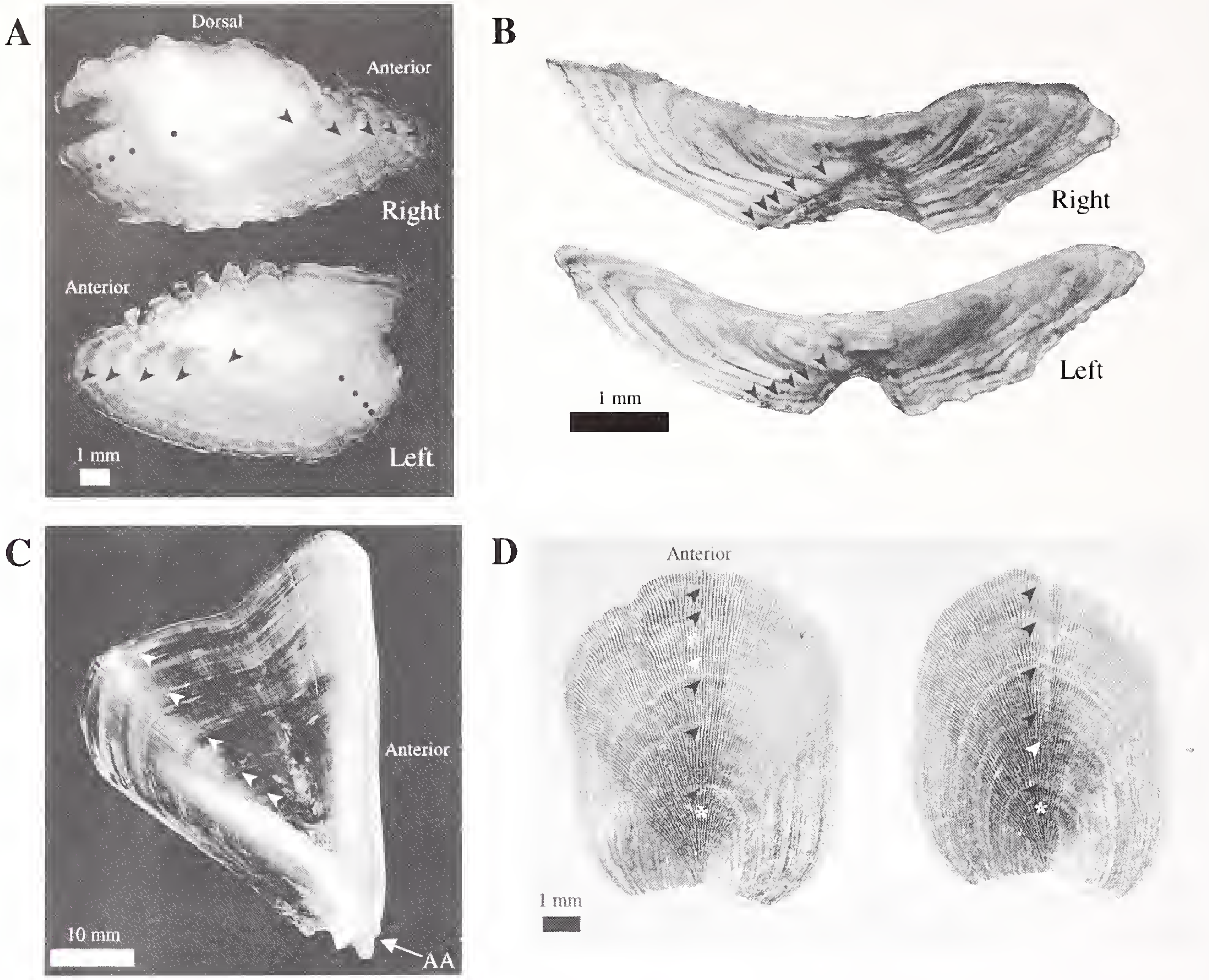

Figure 1

Marks on calcified structures taken from a 5-year-old (determined from sectioned otoliths) female summer flounder, TL = 687 mm, collected in mid-January. Arrows indicate individual marks counted (as described in the "Methods" section). (**A**) Whole otoliths, viewed with reflected light on a black background. Arrows indicate presumed annual marks along the primary counting path; dots indicate presumed annual marks along the secondary path. Whole otolith radial lengths were measured along the primary counting path. (**B**) Transverse otolith sections, viewed in transmitted light. Sectioned otolith radial lengths were measured along the counting path (indicated by arrows) from the center of the focus to the edge along the ventral arm of the sulcal groove. (**C**) Right opercular bone, viewed with reflected light on a black background. AA = articular apex. (**D**) Scale impressions, viewed in transmitted light. White arrows indicate marks that appear on only one of the scales. Asterisks indicate probable false marks. Both scales have a probable false mark prior to the first mark counted.

transmitted light and dark under reflected light, whereas opaque zones appeared dark under transmitted light and white under reflected light. The first presumed annual mark was defined as the first opaque zone after the first translucent zone, where the first translucent zone occupied the central focal area of the opercular bone. Both bones were examined, and the one with the clearest marks was used for aging. Opercular bone radial length (OpRL) was measured to 0.05 mm from the center of the articular apex to the anterior margin edge with calipers.

Scales were soaked in water until flexible and brushed gently with a soft bristle toothbrush. Then 5 or 6 clean, symmetrical, unregenerated scales were dried, taped to an acetate sheet, inserted between two new acetate sheets, and pressed in a Carver laboratory scale press for 2 minutes at 15,000 pounds of pressure and 60°C. Scale impressions were read with a Bell-Howell R753 microfiche reader at 20× and 32×. Presumed annual marks were identified with standard scale reading criteria as described in Smith et al. (1981), Dery (1988), and Almeida et al. (1992). Briefly, readers enumerated marks (Fig. 1D) that exhibited "cutting over" in both lateral fields of the scale that was accompanied by a clear narrow zone in the anterior portion of the scale. Scale radial length (ScRL) was measured to 0.001 mm from the center of the focus to the anterior edge of the scale by using a compound video microscope with the Optimas image analysis system (Media Cybernetics, 1999).

Evaluation of calcified structures

Each structure was examined for age by two readers—twice by reader 1 and once by reader 2. Structures were read in a randomly selected order with no knowledge of fish size or collection date. Ages were assigned on the basis of presumed annual mark counts. Different structures from the same fish were read independently, including right and left otoliths, and at least one week separated the first and second readings of the same structure.

Preliminary evaluations of structures included preparation times, reading times, confidence in the clarity of presumed annual marks, growth of the structures with size of the fish, and agreement in repeated age readings of the same structure (precision). Structures judged acceptable based on those criteria were then evaluated further for agreement in age readings between different structures from the same fish and to see if the number of presumed annual marks increased with structure size and fish size. Our preliminary evaluation indicated otoliths and scales to be superior to opercular bones; therefore opercular bones were not evaluated further.

Preparation time, a measure of the processing efficiency of a structure, was evaluated as the time taken to prepare structures for reading. Clarity of presumed annual marks on a structure was evaluated using both reading times and confidence scores. Reading time was measured as the time taken to read a given structure in an individual fish. Confidence scores, expressed on a scale of 1 (low) to 5 (high), were assigned by the reader to each reading based on the clarity of the marks. Differences in confidence scores between structures were tested at $\alpha = 0.05$ by using the normal approximation to the Mann-Whitney test for ordinal data (Zar, 1996).

The assumption that structure growth is directly related to fish growth was evaluated using regression analysis (Zar, 1996). Structure sizes (ScRL, OpRL, WOTL, WORL, SORL) were regressed on fish TL to determine if the relationships were significant and increasing. Sample sizes varied in these regressions, and in regressions of the number of presumed annual marks on structure size described below because some structures were broken in preparation and could not be measured.

Precision in age determinations for a given structure was evaluated using simple percent agreement in repeated readings within and between readers. Within-reader agreement compared the first and second readings by reader one, and between-reader agreement compared the first readings of each of the two readers. Reader comments on structure features were evaluated to determine the proximal causes of disagreements.

Scales that disagreed in the initial two readings by reader 1 were reread independently a third time by reader 1 to reach a consensus for use in between-structure comparisons. Likewise, right and left otoliths that disagreed in the initial two readings by reader 1 were read a third time to reach a consensus. Structures that showed no agreement in three readings (1 of 81 for scales, 1 of 81 for sectioned otoliths) were not included in between-structure comparisons.

Agreement in presumed annual mark counts between different structures of an individual fish was evaluated by using simple percent agreement between structures and simple linear regression procedures. For the regressions, ages determined by one structure were regressed on ages determined by another structure, and the slope of the regression line was tested to see if it differed significantly from one. A slope of one implies that $y = x$ and that the two structures give the same age. For each regression, we used as the x-variable the structure judged to be superior in the preliminary evaluations.

The assumption that the number of presumed annual marks on a structure is directly related to structure size and to fish size was evaluated using regression analysis (Zar, 1996). The number of presumed annual marks on a structure was regressed on structure size (ScRL, WOTL, WORL, SORL) and on fish TL to determine if the relationships were significant and increasing.

Results

Comparative appearance of calcified structures

All four calcified structures showed concentric marks that were interpreted as annual (Fig. 1). However, these structures differed greatly in the clarity of presumed annual marks.

Presumed annual marks on both whole and sectioned otoliths (Fig. 1, A and B) were typically clear, consistent, and easy to interpret, especially for sectioned otoliths. The right-left difference in the location of the focus had moderate effects on mark clarity for both whole and sectioned otoliths, as described below. Whole otolith marks were most easily read at younger ages, but age had little effect on sectioned otolith mark clarity. The few disagreements in otolith ages were primarily caused by an early, presumably false, mark that often occurred prior to the first presumed annual mark (Fig. 2). This early mark appeared as a thin opaque band close to, but distinct from, the focus and was found on both young (Fig. 2A) and older (Fig. 2B) fish. We tried not to count this early mark in our age readings, because it did not occur consistently in all fish. Finally, only one otolith of 81 pairs was poorly calcified and unable to be read whole, although its age was easily determined upon sectioning.

Presumed annual marks on opercular bones (Fig. 1C) were fairly clear in some fish, but they were more often poorly defined, inconsistent, and difficult to follow across the structure, making age interpretation difficult and highly subjective. Opercular bones commonly exhibited unclear transitions from translucent to opaque zones; the first one or two marks were particularly difficult to distinguish, even on young fish. Zone transitions were often easier to interpret towards the edge of the structure in older fish, although this too varied greatly from fish to fish. The example in Figure 1C is unusually clear and easy to read.

Presumed annual marks on scales (Fig. 1D) were clearer than those on opercular bones, but they still required much subjective interpretation. Figure 1D shows some of the common problems encountered with scales, including presumably false marks (asterisks) and marks that were present on only some scales from the same fish (white arrows). In addition, many fish had regenerated, asymmetrical, or otherwise damaged scales, making it difficult and time-consuming to choose acceptable scales to press. For example, about 20 scales were pressed in order to obtain two scales that were adequate to show in Figure 1D. Interpretation of age from scales of older fish was extremely difficult because marks at the scale edges were often obscured or crowded together, particularly in the narrow lateral fields. Finally, a major source of disagreement in age determination from scales resulted from an early, presumably false, mark that often occurred prior to the first presumed annual mark (Fig. 1D, asterisk). Because this early mark did not appear consistently in all fish or even on several scales from the same fish, we tried not to count it in our age readings.

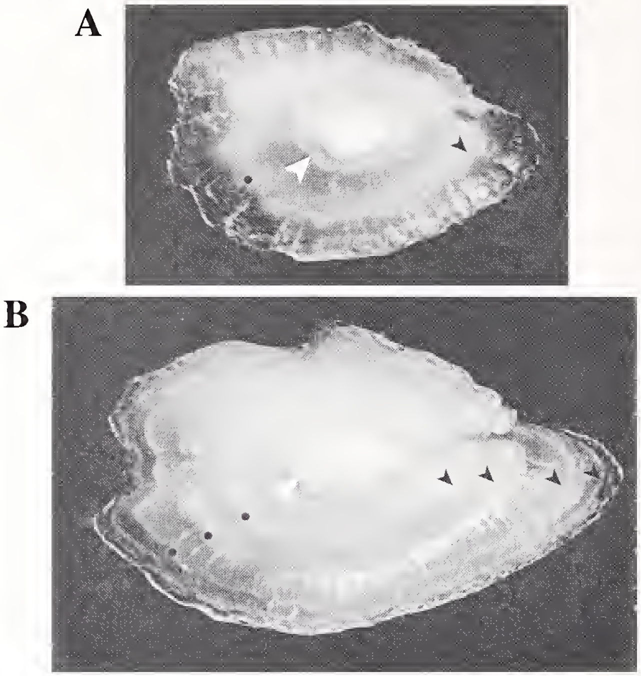

Figure 2

Right whole otoliths showing an early, presumably false, mark. (**A**) is from a 299-mm-TL age-1 fish collected in September, and (**B**) is from a 442-mm age-4 fish collected in October. White arrows point to the early marks. Black arrows indicate primary counting path (anterior field), dots indicate secondary counting path (posterior field).

Preparation times, reading times, and confidence in clarity of marks

Preparation times were short and reasonable for all structures, at less than 15 minutes per fish. Whole otoliths took by far the shortest time because no preparation was required before reading (Table 1). Sectioned right otoliths and opercular bones required 4 to 6 minutes to prepare, whereas scales and sectioned left otoliths took much longer to prepare, about 11 and 14 minutes, respectively. Left sectioned otoliths took much longer to prepare than right sectioned otoliths primarily because they broke much more frequently during sectioning.

Reading times were short and reasonable for all structures, at less than three minutes per fish. Sectioned right otoliths had by far the shortest reading time, at only 0.27 minutes per fish (Table 1). Whole otoliths and sectioned left otoliths had the next shortest reading time, at only about 0.4 to 0.6 minutes per fish. Scales (1.2 min) and opercular bones (2.4 min) both required much more reading time than otoliths, indicating that otoliths could be aged more easily.

Reader confidence scores varied greatly between structures. Sectioned otoliths had by far the highest confidence scores, with values of 4.9 and 4.8 for the right and left, respectively (Table 1). Whole otoliths had somewhat lower confidence scores, with values of 4.1 and 3.8 for the right and left, respectively. Confidence scores were much lower for scales (3.2) and especially for opercular bones (2.3), indicating that these structures were not as easily interpreted. All confidence scores were significantly different from one another (Z=2.10 to 4.18; P<0.0001 to 0.013; individual values not reported).

Regression of structure size on fish size

All calcified structures grew in size as summer flounder body length grew, indicating that each structure could be useful for back-calculation studies. All regressions of structure size on total length were significant at $P < 0.001$, and all slopes were positive (Table 2). All regressions were strong and explained much of the variation in structure size, generally 90% or more, with coefficient of determination values (100 r^2) ranging from 72% to 98%. Values for 100 r^2 were less than 91% only for right and left sectioned otoliths, which were 72% and 85%, respectively.

Agreement in age determinations for the same structure

Agreement (precision) between repeated age readings varied greatly between calcified structures. Precision by the same reader was highest by far (95% to 97%) for sectioned right and left otoliths and left whole otoliths (Table 3). Precision was somewhat lower in right whole otoliths (89%) than in

Table 1

Average preparation times (min), reading times (min) ± standard error (SE), and confidence scores (±SE) for summer flounder calcified structures.

Structure	Preparation time	Reading time	Confidence score
Opercular bones	4.63	2.43 ±0.20	2.31 ±0.16
Scales	10.50	1.20 ±0.13	3.21 ±0.15
Sectioned otoliths			
Right	5.86	0.27 ±0.04	4.91 ±0.04
Left	13.93	0.57 ±0.09	4.75 ±0.05
Whole otoliths			
Right	0.00	0.45 ±0.06	4.10 ±0.11
Left	0.00	0.41 ±0.04	3.84 ±0.10

left whole otoliths; however this could be attributed to the reader learning to use reflected lighting more effectively during the second reading, because 7 of the 9 consensus readings for right otoliths agreed with the second reading. Within-reader agreement was lower with the use of scales (80%), but precision varied with age. Agreement in repeated scale readings was actually high for ages 0 to 4 (92%, n=52), but it decreased to only 59% for fish over age 4 (n=29). Precision was lowest by far in opercular bones (46%), where there were no patterns in agreement by age. Because opercular bones showed the lowest precision and the poorest mark clarity, we did not include them in further evaluations.

Agreement in age determinations between readers also varied greatly among calcified structures. Precision between readers was highest by far (96%) for right sectioned otoliths (Table 3). Agreement was somewhat lower (86% to 88%) for left sectioned otoliths and whole otoliths. Agreement was lowest by far for scales (58%), reflecting the overall poor clarity of marks and the resulting subjectiveness in scale age readings compared with otolith age readings.

Comparison of right and left otoliths

Differences in right and left radial lengths were observed for both whole and sectioned otoliths. The right radial length was significantly shorter than the left in whole otoliths (paired t=17.59, df=73, P<0.0001; Fig. 1A). However, for sectioned otoliths, the right radial length was significantly longer than the left (paired t =–11.72, df=43, P<0.0001; Fig. 1B) because the right otolith is thicker at the focus, where the transverse cross section was taken.

Right and left whole otoliths generally gave the same age readings. Reader one had high age agreement between right and left whole otolith readings (96%), and the null hypothesis that the slope of the line equals one was not rejected (P=0.077, Fig. 3A).

Although right and left whole otoliths generally indicated the same age, they differed in mark clarity. When the posterior field (secondary counting path) was used to verify or determine the number of presumed annual marks, the right otolith was generally much easier to read than the left because of the greater distance between the focus and the posterior margin on the right otolith (Fig. 1A). This greater distance made the marks further apart and more easily distinguishable on the right than on the left otolith. The difference in mark clarity was greatest for older fish and was also reflected in significantly higher confidence scores for the right whole otolith than for the left (Table 1).

Right and left sectioned otoliths also generally gave the same age readings. Reader one had high age agreement between right and left sectioned otolith readings (94%), and the null hypothesis that the slope of the line equals one was not rejected (P=0.393, Fig. 3B).

Table 2

Regression statistics for relationships between structure size and summer flounder total length (TL). Structure abbreviations are defined in the "Methods" section of the text. n = sample size. All regressions were significant at $P < 0.001$.

Structure	Equation	n	100 r^2
Opercular bones	$OpRL = -2.280 + 0.0772\ TL$	66	98
Scales	$ScRL = -0.348 + 0.0126\ TL$	81	93
Sectioned otoliths			
Right	$SORL = -0.015 + 0.0027\ TL$	66	85
Left	$SORL = 0.015 + 0.0018\ TL$	47	72
Whole otoliths			
Right	$WORL = 0.642 + 0.0089\ TL$	76	91
Left	$WORL = 0.601 + 0.0111\ TL$	77	93
Right	$WOTL = 1.280 + 0.0164\ TL$	76	94
Left	$WOTL = 1.530 + 0.0156\ TL$	77	91

Table 3

Average percent agreement, within and between readers, for presumed annual mark counts on summer flounder calcified structures.

Structure	Within reader	Between reader
Opercular bones	46	—
Scales	80	58
Sectioned otoliths		
Right	97	96
Left	95	88
Whole otoliths		
Right	89	86
Left	97	87

Although right and left sectioned otoliths generally gave the same age, presumed annual marks were usually clearer and easier to interpret on the right otolith. Right sectioned otoliths had a much longer counting path and were therefore easier to age than left sectioned otoliths, where the marks were more crowded and less clearly defined (Fig. 1B). This difference was also reflected in higher confidence scores and lower reading times for the right sectioned otolith than for the left (Table 1).

Comparison of different calcified structures from the same fish

Whole and sectioned otoliths generally gave the same age readings. The number of presumed annual marks on whole and sectioned otoliths showed high agreement (95%), with 100% agreement for fish under age 4 (Fig. 4A). In addition, the null hypothesis that the slope of the line equals one was not rejected (P=0.901).

Although whole and sectioned otoliths generally provided the same age, presumed annual marks were often clearer on sectioned otoliths than on whole ones, especially in older fish, where crowding of marks at the edge of whole otoliths became a problem. This observation is supported by the much higher confidence scores for sectioned otoliths (Table 1). As a specific example, the oldest fish in the comparison showed very clear marks and was aged 10 in every reading using both right and left sectioned otoliths (Fig. 5A), and all confidence scores were 5. Marks were less clear on the whole otolith (Fig. 5B), however, with between 8 and 10 marks counted in different readings, and an average confidence score of only 2.5. In general, the use of sectioned otoliths appeared to greatly increase mark clarity in fish over age 4 or 5.

Scales and sectioned otoliths often did not give the same age readings. Agreement in the number of presumed annual marks on scales and sectioned otoliths was undesirably low, at only 80% (Fig. 4B). In addition, the null hypothesis that the slope of the line equals one was rejected (P=0.047). Scales tended to overage compared with sectioned otoliths in fish age 4 and younger, but to underage in fish older than age 4. Agreement between scales and sectioned otoliths was fairly high for ages 0 to 4 (86%, n=56) but decreased to only 65% in fish over age 4 (n=23).

Scales and whole otoliths often did not give the same age readings. Agreement in the number of presumed annual marks on scales and whole otoliths was also undesirably low, at only 76% (Fig. 6). In addition, the null hypothesis that the slope of the line equals one was again rejected (P=0.039). As with sectioned otoliths, scales tended to overage compared with whole otoliths in fish age 4 and younger and to underage in fish older than age 4. Agreement between whole otoliths and scales was fairly high for ages 0 to 4 (85%, n=53) but decreased to only 56% in fish over age 4 (n=25).

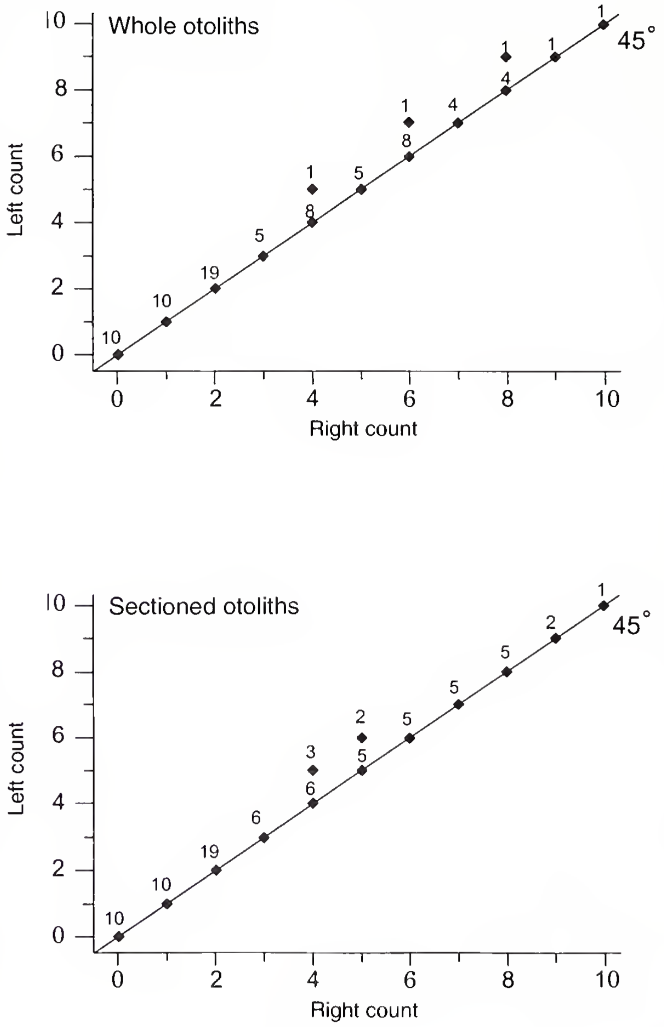

Figure 3

Comparisons of presumed annual mark counts on the left otolith with mark counts on the right otolith for whole otoliths and sectioned otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Increase in number of marks with structure size and fish size

Mark counts on calcified structures increased as structure size and fish size increased, indicating that each structure tested could be useful in age determination. All regressions of mark counts on structure size were significant at P<0.001, and all slopes were positive (Table 4). Regressions were generally strong and explained much of the variation in mark counts because 100 r^2 values were high, generally from 80% to 86%. Values for 100 r^2 were lowest for left sectioned otolith radius and scale radius, at 67% and 73%, respectively. Likewise, all regressions of mark counts on fish size were significant at P < 0.001, and all slopes were positive (Table 5). All regressions were again strong, with 100 r^2 values from 83% to 86%.

Discussion

Comparative evaluation of sectioned otoliths

Our findings indicate that sectioned otoliths are the best structure for aging summer flounder over the age range 0 to 10 years. Sectioned otoliths had the shortest reading times, the highest confidence scores, the highest within- and between-reader agreement, and they were consistently clearer and easier to read than whole otoliths, scales, and opercular bones. These findings are new for summer flounder because no published studies have used sectioned otoliths to age this species. These findings generally agree, however, with many studies on other species that have found sectioned otoliths to be the best aging structure (for examples, Beamish, 1979; Chilton and Beamish, 1982; Beamish and McFarlane, 1983; Lowerre-Barbieri et al., 1994).

Right sectioned otoliths were generally superior to left sectioned otoliths. Although we found high agreement in age between right and left sectioned otoliths, right otoliths were much easier to prepare, and they had a larger counting path, which made it easier to identify the marks, resulting in shorter reading times, higher confidence scores, and higher reader agreement.

Although we have found sectioned otoliths to be the best structure for determining the age of summer flounder, our studies have not proven their accuracy. To do so would require known-age methods or at least marginal increment methods. However, until validation is done, we feel there is sufficient evidence to recommend that sectioned otoliths replace the current practice of using scales for aging summer flounder.

Comparative evaluation of whole otoliths

Our findings indicate that whole otoliths are the second best structure for aging summer flounder over the age range of 0 to 10 years. Whole otoliths had no preparation time and had the second shortest reading times, the second highest confidence scores, the second highest within- and between-reader agreement, and the highest agreement with sectioned otoliths. Whole otoliths were generally easy to read in fish less than age 4 or 5, and we feel they are adequate for these younger ages, especially in large-scale production aging where preparation time is important.

We found that the right whole otolith was often easier to read than the left when the secondary counting path was used. Therefore, although former studies have used the left whole otolith only (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977; Powell, 1982), we suggest that the right should be included in future work.

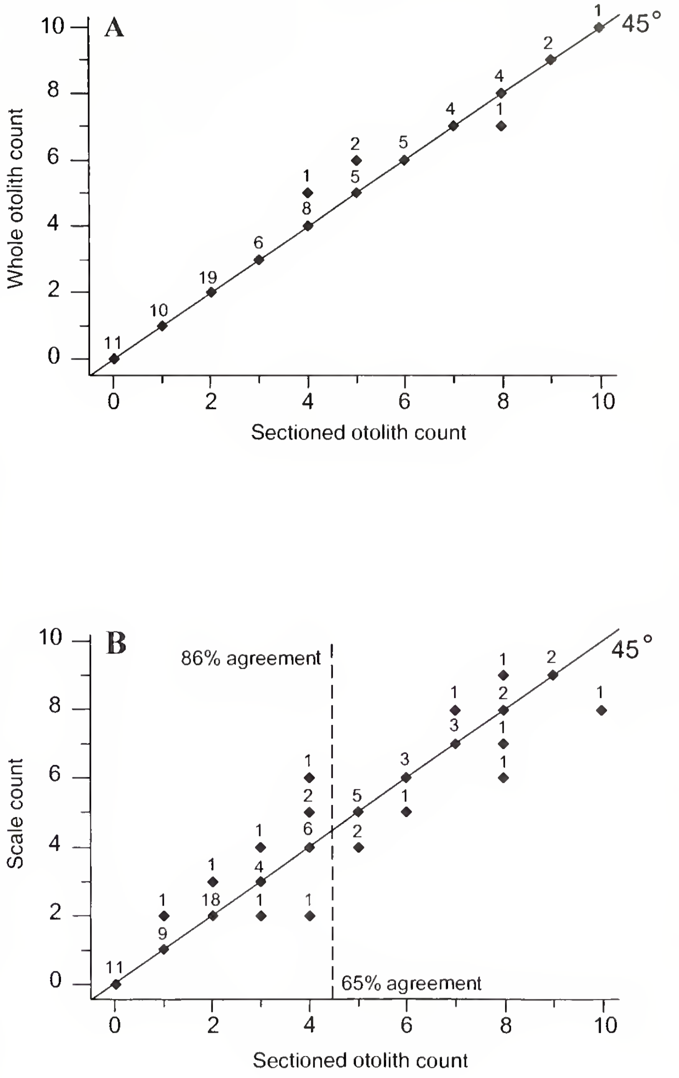

Figure 4

Comparisons of presumed annual mark counts on whole otoliths (**A**) and scales (**B**) with mark counts on sectioned otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Our findings on preparation and reading times, confidence scores, within- and between-reader agreement and agreement with sectioned otoliths are generally new because the literature has not reported detailed evaluations of whole otoliths in summer flounder. Given our findings, we do not agree with the current preference for using scales rather than whole otoliths in summer flounder. Indeed, we disagree with the original reasons for rejecting otoliths, which included 1) poor calcification and poor contrast between opaque and translucent zones (Shepherd, 1980; Smith et al., 1981; Dery, 1988), 2) obscurement of the first mark as the fish ages (Powell, 1982), 3) deviation from the generalized pattern of opaque and translucent zone for-

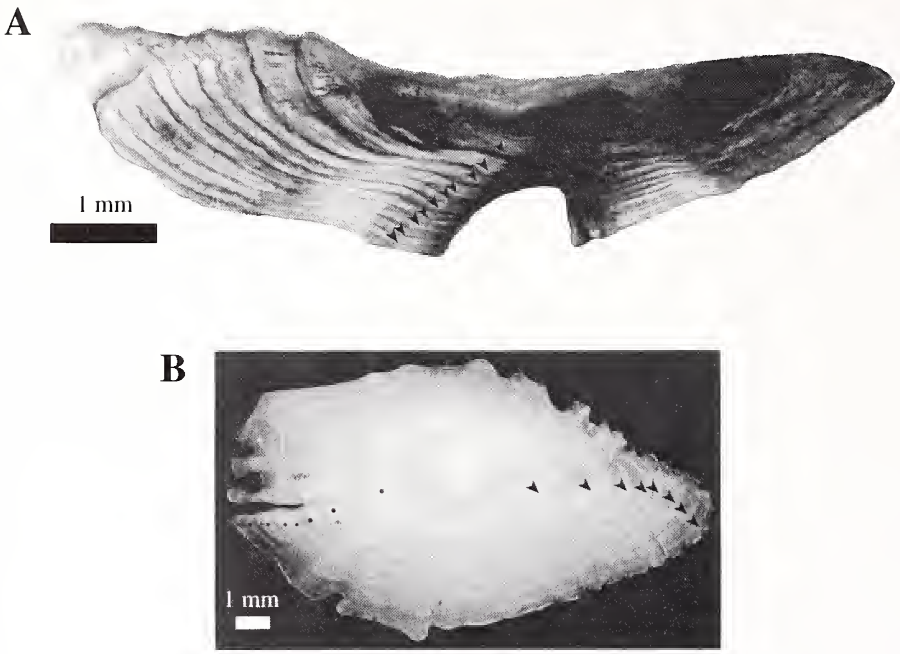

Figure 5

Right sectioned (**A**) and whole (**B**) otolith from a female summer flounder, 10 years old (determined from sectioned otoliths) and 758 mm TL, collected in November. Arrows on the sectioned otolith indicate presumed annual marks. On the whole otolith, arrows indicate primary counting path (anterior field), dots indicate secondary counting path (posterior field). Ten marks are visible in the posterior field of the whole otolith, but only eight marks are visible in the anterior field.

Table 4

Regression statistics for relationships between the number of marks (*Marks*) and calcified structure size for summer flounder. Structure abbreviations are defined in the "Methods" section of the text. n = sample size. All regressions were significant at $P < 0.001$.

Structure	Equation	n	$100\ r^2$
Scales	*Marks* = –2.64 + 1.080 *ScRL*	80	73
Sectioned otoliths			
Right	*Marks* = –3.39 + 5.424 *SORL*	65	80
Left	*Marks* = –3.36 + 6.996 *SORL*	46	67
Whole otoliths			
Right	*Marks* = –4.56 + 1.664 *RWOR*	75	85
Left	*Marks* = –4.47 + 1.367 *LWOR*	76	86
Right	*Marks* = –4.80 + 0.919 *RWOT*	75	86
Left	*Marks* = –4.80 + 0.934 *LWOT*	76	82

Table 5

Regression statistics for relationships between the number of marks (*Marks*) on calcified structures and summer flounder total length (*TL*). All regressions were significant at $P < 0.001$, and sample sizes were 80 fish.

Structure	Equation	$100\ r^2$
Scales	*Marks* = –3.69 + 0.0151 *TL*	83
Sectioned otoliths	*Marks* = –3.86 + 0.0155 *TL*	85
Whole otoliths	*Marks* = –3.90 + 0.0157 *TL*	86

mation in temperate fishes (Smith et al., 1981), and 4) a narrow opaque zone as compared to the translucent zone (Smith et al., 1981). We address these issues in turn below.

We rarely observed poor calcification or poor contrast between opaque and translucent zones of whole otoliths. Rather, our procedures gave good contrast between opaque and translucent zones, so that we had high confidence in our age readings. In addition, we found only one otolith of 81 pairs to be poorly calcified. This otolith was easily aged once it was sectioned, and its pair was not poorly calcified and was aged with high confidence.

We saw little evidence that the first mark becomes obscured at older ages on whole otoliths, as indicated by our high agreement between whole and sectioned otoliths. The hypothesis that the first mark becomes obscured was based on overlap in back-calculated sizes at the second and third marks on whole otoliths (Powell, 1982). However, size in any year class can vary greatly because summer flounder spawn over a protracted season (Smith, 1973; Morse, 1981; Able et al., 1990). Therefore, fish in adjacent year classes can be expected to overlap in size, and Powell's results do not necessarily mean that the first mark becomes obscured with age.

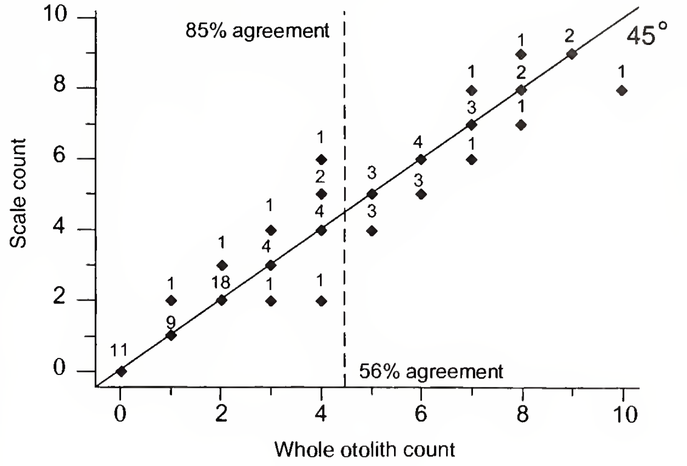

Figure 6

Comparison of presumed annual mark counts on scales and whole otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Smith et al. (1981) reported that summer flounder otoliths deviated from the general pattern of opaque and translucent zone formation seen in other temperate fishes and suggested that opaque zones formed in fall–winter, the reverse of the usual spring–summer formation in other temperate species. We saw no evidence of this reversal. Our fish were collected from October through December, so we should have observed opaque edges on the otolith if the timing of mark formation were reversed from other temperate fishes. Instead, we observed relatively wide translucent zones on the otolith edges. In addition, other studies have not found a reversal in the time of mark formation (Poole, 1961; Powell, 1982; Wenner et al., 1990), and Desfosse (1995) found that opaque zones appeared to form on whole otoliths at approximately the same time as scale marks (May through July). Finally, Smith et al. (1981) presented no data to support their hypothesis that opaque zones formed in the fall and winter. Indeed, their Figure 5 shows an opaque edge on a whole otolith from a summer flounder captured in June.

In agreement with studies in other species (see references below), we found the translucent zone to be wider than the opaque zone on summer flounder otoliths. Smith et al. (1981) felt this was an anomalous occurrence and used it to reject whole otoliths. We disagree with their analysis, however, because many other fishes in our study area, including Atlantic croaker (Barbieri et al., 1994a), weakfish (Lowerre-Barbieri et al., 1994), and Spanish mackerel (Gaichas, 1997) have otoliths with a wide translucent zone and a narrow opaque zone. Such a pattern reflects the fact that opaque zones form over a short time period in these species: April–May in Atlantic croaker and weakfish (Barbieri et al., 1994a; Lowerre-Barbieri et al., 1994) and May–June in Spanish mackerel (Gaichas, 1997). In addition, although the sample size was limited (n=93), Desfosse (1995) found evidence, using marginal increments, that opaque zone formation on summer flounder otoliths occurs over a similarly short time period (May to July). Finally, regardless of whether opaque zones are narrower or wider than translucent zones, otoliths can be used for age determination if the mark can be proven annual.

Comparative evaluation of scales

Our findings indicate that scales are inferior to, and much less desirable than, both sectioned and whole otoliths for aging summer flounder. Scales had significantly lower confidence scores and much higher reading times than sectioned and whole otoliths because marks on scales were often difficult to interpret using objective aging criteria. False marks were common, and different scales from the same fish often indicated different ages. As a result, both within- and between-reader percent agreement and agreement with whole and sectioned otolith age were undesirably low in scales, especially in fish over age 4. We feel that scales should not be used for aging summer flounder if otoliths, especially sectioned otoliths, are available.

The difficulties we found with summer flounder scales generally agree with reports in the literature. Dery (1988), Desfosse (1995), and Bolz et al. (2000), for examples, have reported similar problems interpreting scale marks. Like us, Desfosse (1995) found low within-reader scale agreement (only 46%) in fish over age 4. Desfosse (1995) reported high agreement between scales and whole otoliths (98%) for ages 0 to 5, much higher than the 85% agreement we found for ages 0 to 4. However, 90% of his fish (n=170) were ages 0 to 2 and only one was age 5, a likely explanation for his high percent agreement. Shepherd (1980) reported high agreement (91%) between scales and whole otoliths for moderately old fish (ages 4 to 6), but his sample size was only 21 fish, only one of which was age 6. Our study reported lower overall agreement between whole otoliths and scales (76%), but we examined fish over a much wider age range (ages 0 to 10) than previously reported.

Comparative evaluation of opercular bones

Our comparative studies have found opercular bones to be inferior to both sectioned and whole otoliths in summer flounder, and even to scales. Opercular bones had the lowest confidence scores, the highest reading times, only 46% within reader agreement, and they often exhibited unclear transitions from translucent to opaque zones, particularly at early ages. For these reasons, we feel that opercular bones should not be used for aging summer flounder.

These findings are new for summer flounder, because no previous studies have used opercular bones to age this species. We were disappointed at the poor performance of opercular bones because they have been reported useful in many other species, including perch (LeCren, 1947), carp (McConnell, 1952), yellow perch (Bardach, 1955), northern pike (Frost and Kipling, 1957), tautog (Cooper, 1967; Hostetter and Munroe, 1993), and goldeye (Donald et al., 1992). Many of these studies show photographs of opercular bones with clear, easily recognized marks that have been interpreted as being formed annually. These studies, however, generally have not validated age determination in opercular bones; therefore it is unclear whether they give accurate ages in these other species.

Formation of early marks on otoliths and scales

We sometimes observed an early, presumably false, mark prior to the first presumed annual mark on both otoliths and scales of summer flounder. Although we attempted not to count this early mark, it appeared to be the primary cause for disagreements between our readers in aging both otoliths and scales. This problem has not been reported in summer flounder otoliths, although there is evidence of this early false mark on scales (Dery, 1988; Bolz et al., 2000). Indeed, a primary problem cited by Bolz et al. (2000) for differences in interpretation of summer flounder scales was the choice of a first annual mark.

The early, presumably false, mark that sometimes occurred on summer flounder otoliths and scales appears similar to the first mark reported for Atlantic croaker otoliths (Barbieri et al., 1994a) and might be explained by similarities in certain life history traits of these two species. Both species have a protracted spawning season and spawn over a similar time frame in the Chesapeake Bay region: Atlantic croaker from mid-summer to late fall (Wallace, 1940; Haven, 1957; Barbieri et al., 1994b), and summer flounder from early fall to early winter (Smith, 1973; Morse, 1981; Able et al., 1990). Barbieri et al. (1994a) reported the formation of a first mark on Atlantic croaker otoliths in the first spring following hatching, at 5 to 10 months, with two patterns of early mark formation: 1) the first mark close to, but distinct from, the focus in early hatched fish, and 2) the first mark nearly continuous with the focus in late hatched fish. As with Atlantic croaker, we suggest that the first mark on summer flounder otoliths and scales, which we have referred to as an "early, presumably false, mark," might actually be laid down in the first spring following hatching, at 5 to 8 months, with the same two patterns of early mark formation.

Previous summer flounder aging studies interpret the first annual mark to be laid down on scales and otoliths in the second spring following hatching (Smith et al., 1981; Szedlmayer et al., 1992), at 17 to 20 months, one year after the first annual mark is laid down on Atlantic croaker otoliths. Despite this difference, fish from these two species that are hatched at the same time are currently placed in the same year class. It thus appears that the current age determination methods differ between these two species. For example, according to current conventions (Bolz et al., 2000), a summer flounder hatched in October 2000 would be called age 1 on 1 January 2002, at a biological age of 15 months. This age is several months *before* the first presumed annual mark is laid down on the structures in the second spring following hatching (2002), even though an "early" mark might have been laid down in the first spring following hatching (2001). Similarly, an Atlantic croaker hatched in October 2000 would be called age 1 on 1 January 2002 (Barbieri et al., 1994a), at a biological age of 15 months. However, this age is 8 months *after* the first annual mark is laid down on the otolith, which occurs in the first spring following hatching (2001). Therefore, the two species differ in the way the first annual mark is assigned.

To resolve the issue of early mark formation in summer flounder, we suggest that calcified structures of young-of-the-year fish be examined to determine when the early mark is formed, as Barbieri et al. (1994a) did for Atlantic croaker. Barbieri et al.'s (1994a) validated method automatically assigns an early first mark, formed at 5 to 10 months, to all Atlantic croaker otoliths, whether the mark is distinct or not. If the "early, presumably false, mark" in summer flounder is similar to the first annual mark in Atlantic croaker, an early first mark could likewise be assigned to summer flounder otoliths. If this were done, disagreements on the first mark on summer flounder structures would be fewer, and summer flounder and Atlantic croaker would be aged in exactly the same way. That is, both fish would already have a first annual mark on the structure when ages are advanced to 1 on the 1 January arbitrary birthdate.

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Abstract—A bottom trawl catch of flatfish is composed of fish that were initially in the path of the trawl net and fish that were initially in the path of the bridles and were subsequently herded into the net path. Bridle efficiency (i.e. the proportion of fish in the bridle path that are herded into the net path) for seven species of flatfish was estimated by fitting a model of the herding process to data collected during a field experiment. The experiment consisted of repeatedly making trawl hauls with three different bridle lengths. The model was then fitted to the catch of each species, by length class, as a function of the widths of the net and door spreads at the three bridle lengths. Bridle efficiency was independent of body length for five species (yellowfin sole, *Limanda aspera*; flathead sole, *Hippoglossoides elassodon*; rock sole, *Lepidopsetta bilineata*; Dover sole, *Microstomus pacificus*; rex sole, *Glyptocephalus zachirus*) and ranged from 0.22 for rex sole to 0.40 for rock sole. Bridle efficiency declined with increasing body length for two species (English sole, *Parophrys vetulus*; Pacific sanddab, *Citharichthys sordidus*), ranging from about 0.35 to 0.10 over the lengths sampled.

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Bridle efficiency of a survey trawl for flatfish

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Swept-area estimates of biomass produced by bottom trawl surveys are typically used as relative indices of stock size rather than absolute measures because the sampling efficiency of the trawls[1] is rarely known. As a means to estimate trawl efficiency, Dickson (1993a) developed a model describing the trawling process and the associated fish behavior from an undisturbed state before arrival of a fishing vessel until passage of a trawl codend. For benthic species that remain closer to the sea bottom than the head-rope height, this model specifies that trawl efficiency is primarily determined by two processes: escapement of fish out of the net path by passing either under the footrope or through the mesh (net efficiency) and herding of fish into the net path by the doors, mudclouds, and bridles (bridle efficiency). In our study, we focused on fish herding, considering the problem of quantitatively estimating bridle efficiency or the proportion of fish encountering the bridles that are herded into the net path.

Underwater observations of trawls in operation have revealed that the process of herding differs between semipelagic species, such as Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) that tend to swim near the bottom, and benthic species, such as flatfish that often remain in direct contact with the bottom (Main and Sangster, 1981b). Atlantic cod are apparently stimulated to herd by the sight of the trawl doors and the mudclouds that form in the wake of the doors, whereas flatfish are stimulated to herd by the close proximity or actual touch of the doors and lower bridles as they sweep along the bottom (Hemmings, 1969; High, 1969; Martyshevskii and Korotkov, 1969; Main and Sangster, 1981a, 1981b). Flatfish, once stimulated to move, tend to swim a relatively short distance in a direction perpendicular to the bridles where they slow down or settle on the bottom until the bridle again overtakes them. This pattern is usually repeated, producing what appears to an observer on the trawl headrope as a zigzag path toward the center of the trawl footrope (Main and Sangster, 1981b). While this is occurring, however, flatfish may either swim over the bridle or become exhausted and be overtaken by the bridle and thereby escape the herding process. Bridle efficiency is determined by the relative frequency of such events.

Perhaps because the mechanism of herding seems more obvious for flatfish than other types of fish, the earliest mathematical models of fish herding were based on the contact-swim-contact pattern of flatfish herding (Hemmings, 1969; Foster et al., 1981; Fuwa et al,. 1988; Fuwa, 1989). These models were focused on individual fish and typically considered both the physical aspects of the trawl, such as shape and speed, as well as the biological aspects of the fish, such as size and endurance. Although these models provided a more structured way of examining fish herding and allowed the quantitative evaluation of the relative importance of the various aspects of the process, they were of limited use in estimating bridle efficiency of specific trawls because some of the required parameters were unknown or difficult to estimate.

The approach to modeling fish herding changed markedly after Engås and Godø (1989a) conducted trawling experiments to quantitatively examine the effects of variations in bridle length on catch for each area and fish size. Reasoning that the changes in catch that accompanied the changes in bridle length provided information on the efficiency of herding, Dickson (1993b) estimated the bridle efficiency coefficients for Atlantic cod and haddock by fitting a simple linear model of the trawl herding process to data from Engås Godø's (1989a) experiments. Ramm and Xiao (1995) conducted a similar trawl herding experiment and developed a new type of herding model appropriate for cases in which escapement at the center of the trawl is zero.

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[1] Throughout this paper, we will refer to the trawl as an entire fishing gear comprising the net, bridles, and doors.

In our study, we extended Dickson's (1993a) herding model so that it is more specific to the peculiarities of flatfish herding and additionally extended Dickson's (1993b) approach to fitting the model to data by providing a more rigorous statistical foundation. The model was then applied to herding data for the 83-112 Eastern bottom trawl (Armistead and Nichol, 1993) used by the Alaska Fisheries Science Center (AFSC) to conduct its annual bottom trawl survey of the Eastern Bering Sea. These data were collected during two herding experiments, patterned after those of Engås and Godø (1989a), in which emphasis was placed on seven species of flatfish: yellowfin sole (*Limanda aspera*), flathead sole (*Hippoglossoides elassodon*), rock sole (*Lepidopsetta bilineata*), English sole (*Parophrys vetulus*), Dover sole (*Microstomus pacificus*), rex sole (*Glyptocephalus zachirus*), and Pacific sanddab (*Citharichthys sordidus*).

Materials and methods

Development of the herding model

Consider that the area of the bottom swept by a trawl consists of two components, the area between the wingtips of the net (net path), and the area between the wing tips and the doors (bridle path; Fig. 1). The catch (N) obtained in a trawl can be represented as some proportion, k_n, of the number of fish in the net path (N_n) plus some proportion, P, of the number of fish in the bridle path (N_b). If the probability of capture for a fish herded into the net path is identical to that of a fish initially in the net path, then P can be considered equal to $k_n k_b$, where k_b is the bridle efficiency[2] or the proportion of fish within the bridle path that is herded into the net path. Algebraically this is expressed as

$$N = k_n N_n + k_n k_b N_b. \tag{1}$$

The numbers of fish within the net and bridle paths are equal to the fish density (D) multiplied by the path areas, that is,

$$\begin{aligned} N_n &= DLW_n \\ N_b &= DL\,(W_d - W_n), \end{aligned} \tag{2}$$

where L = tow length;
W_n = the width of the net path; and
W_d = the width of the door path (Fig. 1).

Combining all terms, the total catch then can be expressed as

$$N = k_n DLW_n + k_n k_b DL\,(W_d - W_n). \tag{3}$$

At this point the model is quite similar to the one proposed by Dickson (1993a) to describe the trawl catching process for Atlantic cod and haddock. We now modify the model to make it more specific to flatfish by considering that herding is restricted to the portion of the bridle path in which the lower bridle is in contact with the bottom. This can be expressed as

$$N = k_n DLW_n + k_n hDL\,(W_d - W_n - W_{off}), \tag{4}$$

where W_{off} = the width of the path in which the bridle is not in contact with the bottom; and
h = the herding coefficient or the proportion of fish in the bridle contact path that is herded into the net path.

Thus, k_b is the average efficiency over the entire bridle path and h is the average efficiency in the portion of the bridle path where herding actually occurs.

Description of the herding experiments

The strategy that we used in our herding experiments was to repeatedly trawl in such a way that the width of the net path was held approximately constant, whereas the width of the door path was varied among three distances by changing the length of the bridles. The first of two herding experiments was conducted 25 July–2 August 1994 aboard the FV *Arcturus* in the eastern Bering Sea and focused on yellowfin sole, flathead sole, and rock sole. The second experiment was conducted 14–25 September 1994 aboard the RV *Alaska* off the coast of Washington State and focused on English sole, Dover sole, rex sole, and Pacific sanddab. For both experiments, we used a blocked sampling design to minimize the effects on catch of the spatial variation in fish density. In each geographic block, three nearby, but nonoverlapping, 30-min trawl hauls at a speed of 1.5 m/s were made with each of three bridle lengths chosen in random order. Bridles measured 27 m, 55 m (the standard length used on AFSC surveys), and 82 m in length and were constructed of 16-mm diameter steel cable. Tailchains connecting the doors to the bridles (Fig. 1) were always constructed of 13-mm diameter long link chain but differed in length between vessels (Table 1). Trawl doors were always a "V" style measuring 1.8 m × 2.7 m and weighing 910 kg. On all hauls, spreads of the doors were measured at the tops of the wings simultane-

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[2] In Dickson (1993a) this same quantity is referred to as sweep efficiency (k_s). We use the term "bridle efficiency" because the 83-112 Eastern trawl does not have sweeps connecting the bridles to the doors as does the trawl considered by Dickson.

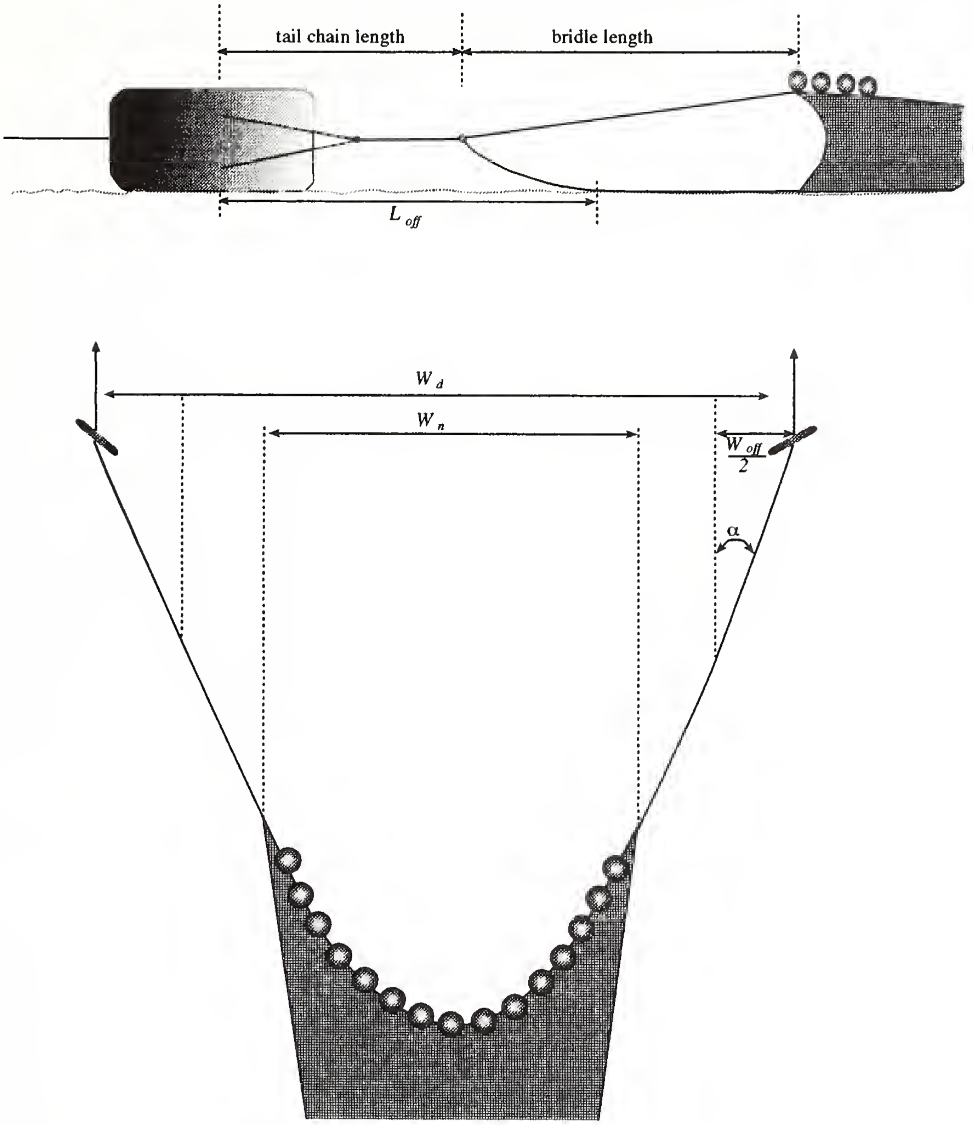

Figure 1

Definitions of the trawl measurements. Included are the tailchain length, bridle length, length of the bridle not in contact with the bottom (L_{off}), width of the door path (W_d), width of the net path (W_n), width of the bridle path not in contact with the bottom (W_{off}), and bridle angle (α).

ously and continuously with an acoustic trawl mensuration system. Tow length was measured as the straight line distance between the GPS positions of first and last footrope contact with the bottom determined with a time-depth recorder attached to the headrope. Bottom temperature was recorded at two second intervals with a bathythermograph and averaged 3.0°C during the eastern Bering sea experiment and 7.0°C in the West Coast experiment. Bottom depth averaged 76 m during the eastern Bering sea experiment and 67 m in the West Coast experiment. The catch from each haul was first sorted to species; then the catch of each species was weighed in the aggregate and fish of each species were individually measured for total length in centimeters. Both experiments were conducted during daylight hours to best approximate the sampling protocol used on most AFSC bottom trawl surveys.

Estimating W_{off}

The herding experiments provided data on N, L, W_d, and W_n, but obtaining data to estimate W_{off} required a different type of experiment. We obtained the necessary data by directly observing the contact of the lower bridle with the bottom on an experimental cruise conducted 9–12 Septem-

Table 1

Trawl configuration parameters for each herding experiment. Included are the bridle and tail chain lengths that were used, the number of sampling blocks occupied, and the means and standard deviations (in parentheses) of the wing spread, door spread, and bridle angle. Also included are the mean values for the bridle off-bottom path width (W_{off}). WA = Washington State.

Experiment	Bridle length (m)	Tailchain length (m)	Number of blocks	Wing spread (m) (W_n)	Door spread (m) (W_d)	Bridle angle (deg) (α)	Bridle off-bottom path width (m) (W_{off})
Bering Sea	27.3	13.9	15	18.0 (0.6)	44.6 (2.1)	18.9 (1.2)	23.3
	54.6	13.9	15	17.3 (0.6)	58.7 (2.9)	17.6 (1.0)	21.8
	81.6	13.9	15	17.1 (0.5)	71.6 (3.7)	16.6 (1.0)	20.5
West Coast (WA)	27.3	8.0	19	17.7 (0.3)	41.9 (0.7)	20.0 (0.5)	24.1
	54.6	8.0	19	17.1 (0.6)	57.5 (1.5)	18.8 (0.7)	23.1
	81.6	8.0	19	16.8 (0.3)	70.0 (1.5)	17.3 (0.5)	21.3

ber 1995 aboard the RV *Alaska* off the coast of Washington State using the 83-112 Eastern trawl equipped with standard 55-m bridles. The experiment consisted of recording views of the lower bridle with a silicon-intensified target (SIT) video camera while the trawl was in operation. The camera was mounted in a positively buoyant case attached to the upper bridle with a 1-m tether line and aligned so that the lower bridle directly below the attachment point could be viewed. The tether was positioned at 5-m intervals between 23 m and 43 m behind the doors. One 15-min tow at each of the five bridle positions was taken at both depths of 20 m and 35 m.

The video tapes were subsequently analyzed to determine the degree of bottom contact at each bridle position. This was done by viewing each tow at ten randomly chosen 10-sec sampling intervals. Bottom contact was recognized by the presence of sediment that is mixed into the water at the point of contact. Because the bottom is irregular, when the bridle has weak contact, just the tops of the irregularities are touched, whereas when the bridle has strong contact, the entire bottom in the field of view is touched. The average percentage of the bridle in the field of view that was in contact with the bottom was scored according to the following four-level scale: 1) no contact, 2) ≤25% contact, 3) >25% and ≤75% contact, 4) >75% contact. The degree of bottom contact at each bridle position and depth was then estimated as the mean of the ten evaluations.

The length of the bridle not in contact with the bottom (L_{off}, Fig. 1) was defined as the distance between the attachment of the tailchain to the door and the point along the bridle where bottom contact was 50%. To estimate L_{off}, a logistic function was fitted to the bottom-contact and bridle-position data with generalized linear modeling (Venables and Ripley, 1994), then the fitted logistic equation was evaluated at a bottom contact score of 3.0 (i.e. bottom contact >25% and <75%). Variance of L_{off} was estimated with bootstrapping (Efron and Tibshirani, 1993), where the bootstrap samples were obtained by randomly choosing, with replacement, from the haul mean scores at each bridle position.

In subsequent analysis, L_{off} was treated as a constant for all three bridle lengths and for both vessels. However the variable actually used in the herding model, W_{off}, was calculated as L_{off} multiplied by the sine of the bridle angle (Fig. 1) and therefore varied slightly between bridle lengths and experiments because the bridle angle varied.

In addition to the camera placements for measuring L_{off}, we also made placements at bridle positions both closer to the doors to examine for any evidence of herding in the area where the lower bridles were not in contact with the bottom and closer to the wing tips to determine if bottom contact was maintained continuously near the junction of the bridle and wing.

Fitting the model to the herding data

The herding model (Eq. 4) was modified in several ways to clarify the way it was fitted to the experimental data and to better define its underlying statistical structure. First, because of the block design of the experiment, fish density (D) was considered to be a constant within each block but to vary between blocks. Furthermore, net efficiency (k_n) was also considered to be a constant for all tows within a block because depth and other bottom conditions are nearly constant but vary between blocks. Because D and k_n are both block-specific constants and are confounded in the model, they were combined into a new constant k. Second, herding can vary with fish length (Engås and Godø, 1989a; Dickson, 1993b), therefore Equation 4 was modified to allow length dependency. The modified equation is

$$N_{ijk} = k_i\left[LW_n\right]_{ij} + k_i h_k\left[L(W_d - W_n - W_{off})\right]_{ij} + \varepsilon \tag{5}$$

where the subscript i = block number;
j = bridle length within a block;
k = fish length class and
ε = ~ $N(0, \sigma)$.

For each size class, fitting Equation 5 to the herding data required estimation of $N+1$ parameters, where N is the number of blocks sampled (i.e. a unique value of k for each block and a common value of h for all blocks). Because the model is nonlinear, because of the product of k and h, it was fitted to the catch and trawl measurement data by using nonlinear regression (Venables and Ripley, 1994). Fish length classes used in the calculations were chosen such that the number of length observations was approximately equal among classes. Ten length classes were usually chosen, but for species with narrow length ranges, as few as eight were used. After fitting the model, estimates of the variances of the parameters were obtained from the inverse of the information matrix (Seber and Wild, 1989).

Bridle efficiency (k_b) for each size class was calculated from the estimated value of the herding coefficient (h) by using the relationship

$$k_b = h\left(1 - \frac{W_{off}}{(W_d - W_n)}\right). \tag{6}$$

Although up to this point h was treated as a constant for all bridle lengths, k_b is not a constant because the proportion of the bridle path in contact with the bottom will vary with bridle length. Therefore k_b was evaluated only for the standard bridle length. Variance of k_b was approximated as

$$Var(k_b) = \left(1 - \frac{W_{off}}{(W_d - W_n)}\right)^2 Var(h). \tag{7}$$

Although the model that we used allows for length-dependent herding, herding may not be a length-dependent process in all species. We performed a simple test of linear length-dependency by regressing the estimated values of k_b on the midpoints of the associated length intervals. For species in which the slope was not significantly different from zero, the herding model was refitted to the data with all length intervals combined. For species in which the slope differed significantly from zero, we calculated a functional relationship between k_b and fish length. Among the possible relationships examined, the best fit was obtained with the exponential model

$$k_b = a + b\exp(cL), \tag{8}$$

where a, b, c are parameters; and
L = the midpoints of the length classes.

Model fitting was done by using nonlinear regression. Variance of the predicted value of k_b was estimated with bootstrapping (Efron and Tibshirani, 1993), where the bootstrap samples were obtained by sampling the residuals from the regression, with replacement, and adding them to the predicted values of k_b. The functional relationship between the standard deviation of k_b and fish length was approximated by using a parabolic model fitted by linear regression.

One implicit assumption of the herding model is that h is constant for all three bridle lengths. We tested the validity of this assumption by comparing the goodness of fit of a modified version of the herding model to that of the original. In the modified model, the herding coefficient was represented as

$$h_l = h + dL,$$

where h_l = the herding coefficient at bridle length L;
h = the mean herding coefficient for all bridle lengths;
d = a free parameter; and
L = a coded value of bridle length equal to −1, 0, 1 for the short, medium, and long bridles (with this notation, negative values of d indicate that h_l declines with increasing bridle length).

Because this is a comparison of two nonlinear models with differing numbers of parameters, we used as a measure of fit the penalized sum of squares; that is, RSS/(n–$2p$), where RSS is the residual sum of squares, n is the number of data values, and p is the number of parameters (Hilborn and Mangel, 1997, page 115). A linear change in h_l is considered significant when the penalized sum of squares for the modified model is less than that for the original model.

Results

Herding experiments

Fifteen blocks were completed in the Bering Sea herding experiment; 19 blocks were completed in the West Coast herding experiment. Although the intention of the experiments was to maintain a constant trawl geometry at all bridle lengths, in both experiments wing spread decreased 0.9 m and bridle angle decreased an average of 2.5% from the shortest to the longest bridles (Table 1). The experimental change in bridle length had a pronounced effect on the area swept by the bridles. In the Bering Sea experiment, for example, total bridle length (i.e. bridle plus tail-chain length) was increased by 143% from the shortest to the longest bridle, which, in turn, produced a 64% increase in door path width (W_d) and a 113% increase in total bridle path width (W_d – W_n). However, this increase in bridle length also produced a 930% increase in the bridle contact path width ($W_d - W_n - W_{off}$; Table 1) because only a small portion of the bridle remained in contact with the bottom at the shortest bridle length.

Catch per area swept by the net increased significantly with increasing bridle length for six of the seven species (rock sole, yellowfin sole, flathead sole, Dover sole, English sole, rex sole; weighted linear regression, $P<0.010$) indicating strong herding by the bridles (Fig. 2). For Pacific sanddab, however, the increase was not significant ($P=0.096$).

Fish length changed significantly ($P<0.05$) with increasing bridle length for only two species (English sole and Pacific sanddab; Table 2) and in both cases smaller fish were

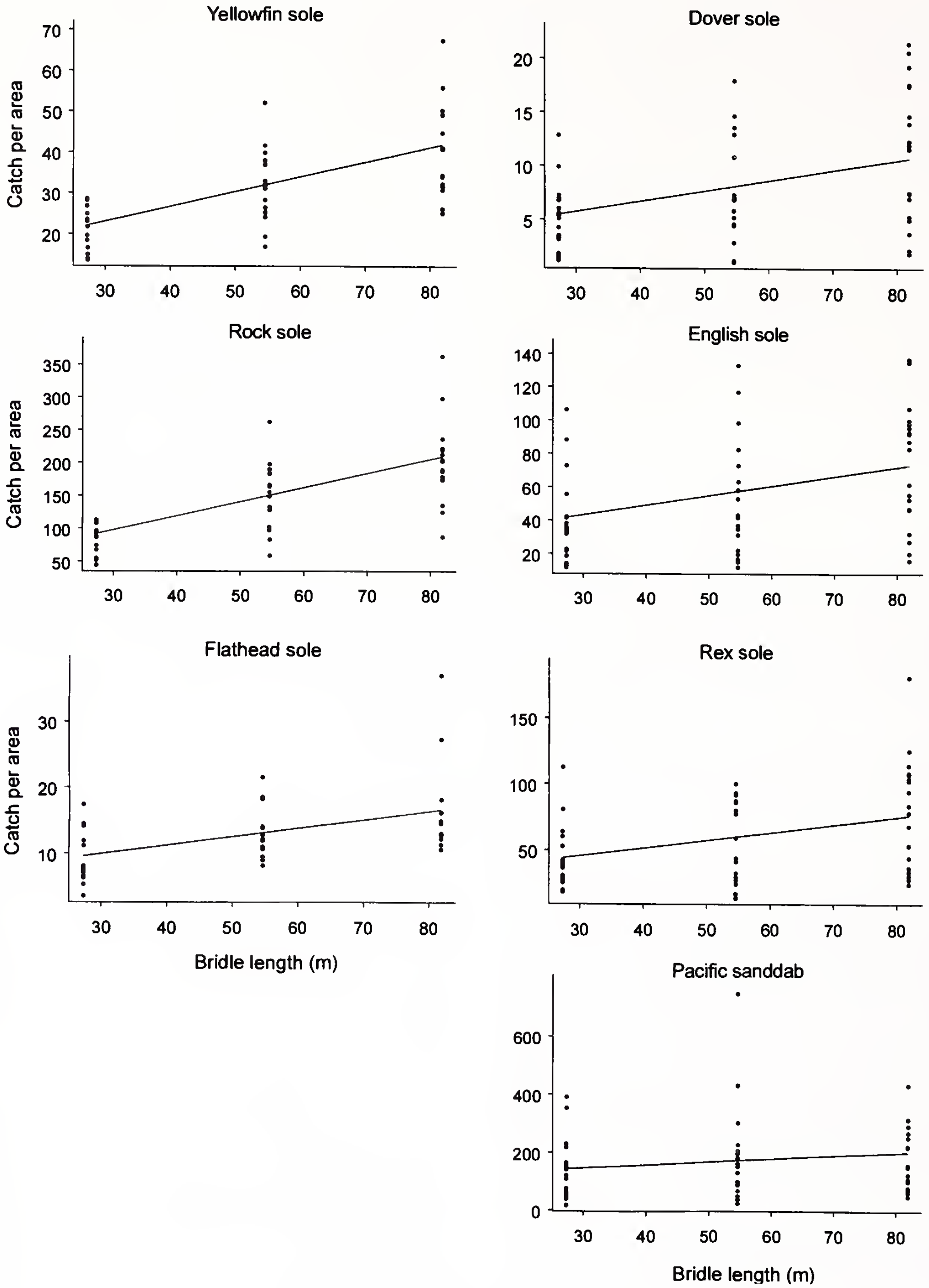

Figure 2

Catch in numbers per hectare swept by the net (i.e. tow length multiplied by net width), by species, plotted against the bridle length in meters. A straight line, fitted to the data with weighted (inverse of the variance) linear regression, is also shown for each species. Significance of the slope of this line indicates that herding contributes to the catch.

caught at longer bridle lengths. Because four of the remaining five species also displayed declining, but nonsignificant, changes in fish length, a decrease in fish length with increasing bridle length seems to be a general flatfish pattern for this trawl. In all cases, however, the magnitude of the decrease was quite small (Fig. 3).

Table 2

Relationship of mean fish length to increasing bridle length. The total number of fish measured, the slope of the regression line fitted to mean length at each tow versus bridle length, and the probability that the slope equaled zero is shown for each species. Only English sole and Pacific sanddab are significant. Note that a negative slope indicates that smaller fish are caught with longer bridles.

Species	Number of fish measured	Slope	P(slope=0)
Rock sole	23,586	–.075	0.23
Yellowfin sole	7231	0.008	0.83
Flathead sole	2927	–0.311	0.31
Rex sole	22,774	–0.052	0.56
Dover sole	12,507	–0.036	0.75
English sole	21,968	–0.277	<0.01
Pacific sanddab	34,209	–0.383	<0.01

Table 3

Regression of k_b on the midpoints of fish-length classes. The number of length classes, the slope of the regression line, and the probability that the slope equals zero are shown for each species. Only English sole and Pacific sanddab have significant ($P<0.05$) slopes.

Species	Number of size classes	Slope	P(slope=0)
Rock sole	10	–0.006	0.088
Yellowfin sole	10	0.001	0.767
Flathead sole	10	–0.004	0.249
Rex sole	10	–0.004	0.354
Dover sole	9	–0.003	0.564
English sole	8	–0.027	0.006
Pacific sanddab	10	–0.016	0.008

Estimating W_{off}

The degree of contact of the lower bridles with the bottom varied with increasing distance from the doors. Contact never occurred in the first 28 m (Fig. 4). At greater distances, contact became intermittent and was focused primarily at the tops of sand ripples. Contact reached 50% at 36 m and nearly 100% at 40 m (Fig. 4). The estimated value for L_{off} was 36.0 m (95% confidence intervals; ±1.4 m). This value was assumed to be constant for all three bridle lengths and both vessels.

Fit of the model

Of the seven species examined for length-dependent herding, only Pacific sanddab and English sole had significant slopes in the regressions of k_b on fish length (Table 3, Fig. 5). In both cases the slope was negative indicating that herding efficiency decreased with increasing fish length.

For the five species displaying no significant length-dependent herding, the estimated herding efficiency in the bridle contact path (h) varied from 0.50 for rex sole to 0.84 for rock sole, whereas the herding efficiency in the entire bridle path (k_b) varied from 0.22 to 0.40 (Table 4). For the two species showing significant length-dependent herding, English sole and Pacific sanddab, k_b varied from about 0.35 at the shortest lengths to about 0.08–0.10 at the longest lengths (Fig. 5).

Test of model assumptions

For six of the seven species, the fit of a modified herding model, with h parameterized as a linear function of bridle length, produced a larger penalized sum of squares than the fit of the original model with a constant h. This finding indicates that the fit was not improved by the specification of a linear variation in h, and that the assumption of constancy of h at all bridle lengths is valid. However, for Pacific sanddab the fit was significantly improved by the modification. Because the sign of the slope parameter of the linear change was negative, it indicates that for this species herding was less effective at longer bridle lengths.

Discussion

Bridle efficiency

Herding by the bridles of the 83-112 Eastern trawl substantially contributes to the catch of flatfish. For rock sole, as an example, approximately 49% of the catch comprised fish that were herded into the net path.[3] This contribution is ignored when swept-area estimates of abundance are based on wing spread rather than door spread. Although such an assumption could lead to an overestimate of abundance, herding is only one component of the trawl catching process and the gains from herding could be offset by the losses due to net escapement.

Bridle efficiency did not vary with fish length for five of the seven species examined (Table 3); consequently, for these species, herding by the bridles is not length selective. A similar lack of length selection was observed by Bridger (1969) in a study examining the herding of lemon sole (*Pseudopleuronectes americanus*). For English sole and Pacific sanddab, however, bridle efficiency clearly declined with increasing fish length and therefore herding is length selective.

Such length selectivity should lead to a change in mean fish length when the average duration of herding experienced by an individual fish is increased by an increase in bridle length. Without exception, the changes in mean fish length that we observed with increasing bridle length are

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[3] From Equation 4, it can be shown that the herded proportion in the catch is equal to $hw_{on}/(w_n + hw_{on})$, where $w_{on} = w_d - w_n - w_{off}$.

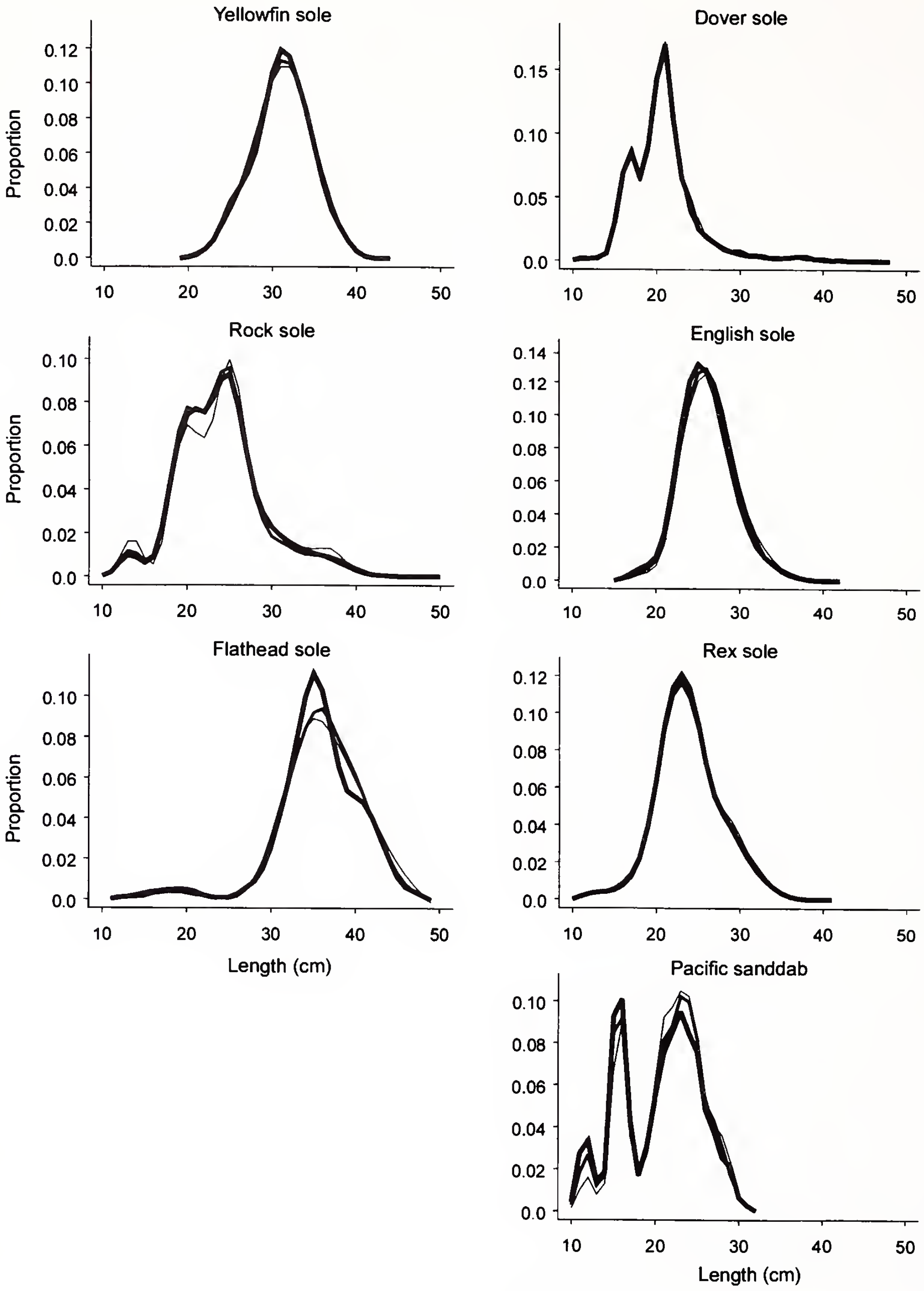

Figure 3

Length-frequency distributions, expressed as proportions, by bridle length. Thin solid lines represent short bridles, medium solid lines represent standard bridles, and thick solid lines represent long bridles.

consistent with the observed changes in bridle efficiency with fish length. All five species with length-independent herding efficiency displayed no change in mean length, whereas both species with herding efficiency that declined with fish length displayed a decline in mean length with increasing bridle length (Tables 2 and 3).

Table 4

Estimates of bridle efficiency, k_b, the standard deviation (SD) of k_b by species, and the r^2 for the model fitted without length dependency. For the two species where k_b varies with length, parameters for predicting k_b and SD(k_b) as functions of fish length are provided. The two functions are $k_b = a + b \exp(cL)$ and SD(k_b)= $a + bL + cL^2$, where L = fish length in centimeters; and h = the average efficiency in the portion of the bridle where herding occurs.

					Parameters of $k_b = f$(fish length)			Parameters of SD(k_b) = f(fish length)		
Species	r^2	h	k_b	SD(k_b)	a	b	c	a	b	c
Rock sole	0.91	0.840	0.400	0.056						
Yellowfin sole	0.77	0.580	0.275	0.057						
Flathead sole	0.70	0.510	0.242	0.065						
Rex sole	0.71	0.502	0.216	0.061						
Dover sole	0.80	0.636	0.274	0.064						
English sole	0.81	0.384	0.165	0.041	0.033	42.667	−0.221	0.3338	−0.0232	0.00042
Pacific sanddab	0.83	0.161	0.070	0.030	0.046	36.783	−0.354	0.0774	−0.0059	0.00012

Form of the herding model

The herding model that we propose is essentially the same as Dickson's (1993a) model except that it includes an additional parameter (W_{off}) needed to correct a potential shortcoming of the original. This shortcoming can be seen in the following example, which for simplicity considers a herding experiment with only two bridle lengths. Starting with Dickson's model (similar to Eq. 3 in our study), an estimator for k_b can be derived from the quotient of the catches obtained with long bridles to the catches obtained with short bridles:

$$k_b = \frac{W_n - RW_n}{R(W_{d1} - W_n) - (W_{d2} - W_n)},$$

where $R = N_2 / N_1$;
W_{d1}, W_{d2} = the door spreads with the shorter and longer bridles; and all other variables are the same as those in Equation 3.

Estimates of k_b, based on values of W_{d1}, W_{d2}, and W_n from the Bering sea experiment, increase with catch ratio (R) and reach a value of 1.0 when R equals the ratio of the door spreads (W_{d2}/W_{d1}). This increase indicates that the bridles are completely efficient (k_b=1.0) when the proportionate increase in the area subjected to herding equals the proportionate increase in catch. If R exceeds the ratio of the door spreads, then k_b>1.0, which is not feasible. In our herding experiments, however, R exceeded door spread ratios for all species except English sole and Pacific sanddab; therefore, in most cases the unmodified herding model failed to provide feasible estimates of k_b. By comparison, estimates of k_b from the modified model are feasible over the entire observed range of catch ratios.

In our initial attempts to fit the herding model to experimental data, we treated W_{off} as an additional parameter to be estimated in the fitting process. However, it soon became clear that W_{off} and h are confounded in the model and that convergent solutions required an independent estimate of W_{off}. We were able to obtain such an estimate and observe fish behavior near the bridle by using video methods, because in the standard configuration of the 83-112 Eastern trawl the bridles are not obscured by mudclouds over much of their lengths. However, in perhaps more typical cases where the bridles are obscured by the mudclouds, estimation of W_{off} with video methods would be problematic. A further complication in such cases is that the mudclouds themselves might provide a herding stimulus and confuse the interpretation of W_{off}.

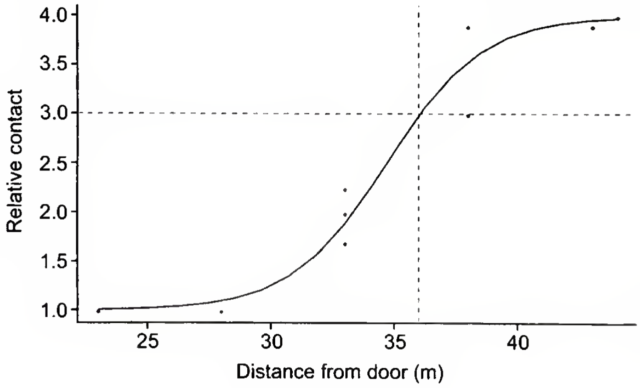

Figure 4

Relative bottom contact of the lower bridle plotted against the distance measured from the door for each camera position and depth. Also shown is the fit of a logistic equation. L_{off} is defined as the distance (vertical dotted line) corresponding to a relative bottom score of 3.0 (horizontal dotted line).

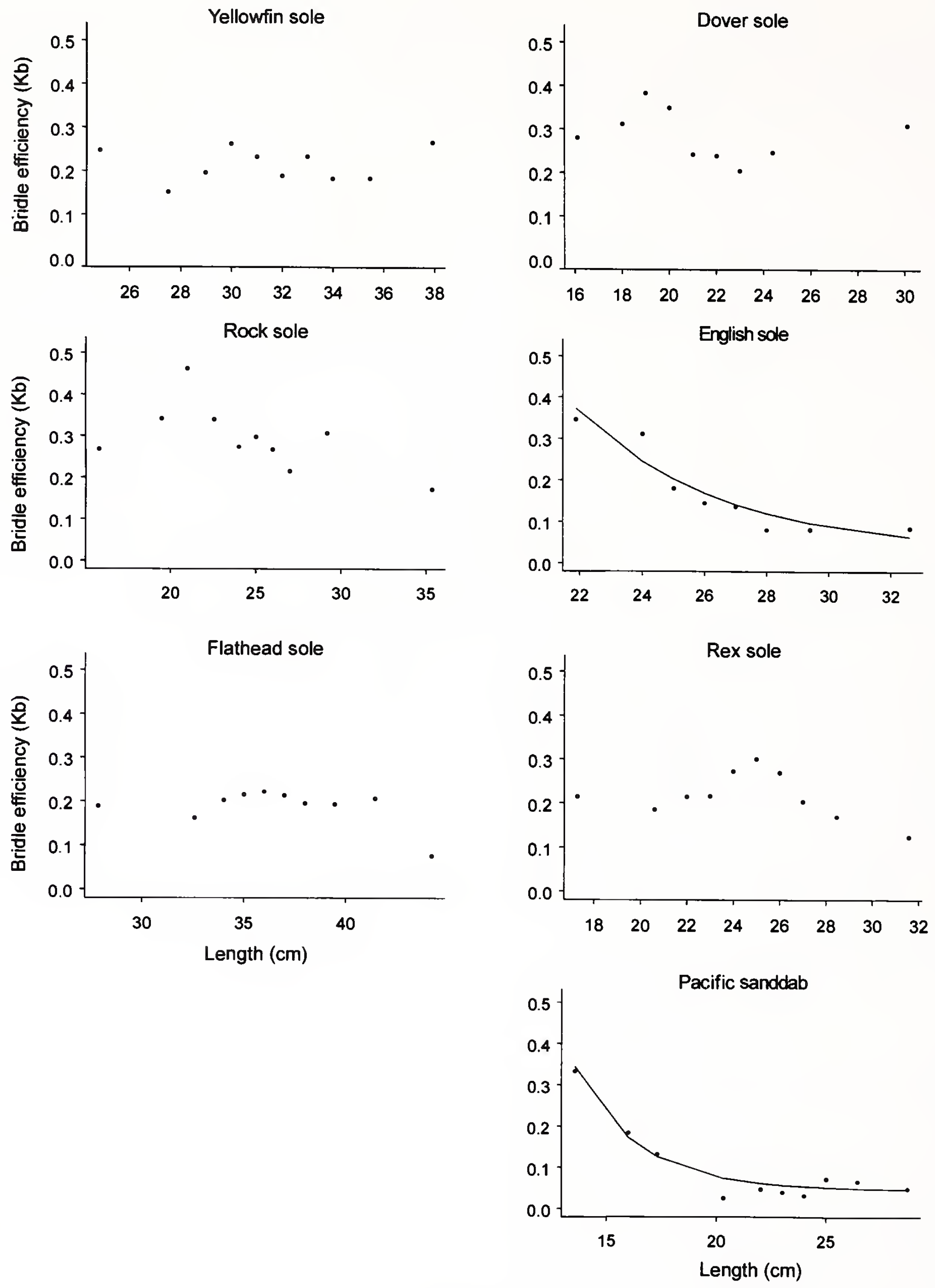

Figure 5

Estimated bridle efficiency (k_b) plotted against fish length in centimeters. The fit of an exponential model (Eq. 8) to the data is also shown for English sole and Pacific sanddab, the two species with significant decreases in k_b with increasing length.

Model assumptions

The adequacy of the herding model and the appropriateness of our herding efficiency estimates rest on a variety of assumptions. First, we assumed herding efficiency was independent of bridle length. If, however, exhaustion was a primary determinant of bridle efficiency, then it is possible that individual endurance would be exceeded more frequently at longer bridle lengths and would thereby diminish herding efficiency. This assumption was tested by comparing the fit of a herding model with a bridle-length dependent h to that of an unmodified model. The modified model provided a better fit for only one species, Pacific sanddab. Because this species is the smallest of the seven (Fig. 3), it is likely that its swimming endurance might have been exceeded at the longest bridle length. However, for the remaining species the assumption is valid.

Second, we assumed that none of the fish in the area spanned by W_{off} are herded into the net path. Although we recognize that the doors and the mudclouds immediately behind the doors must herd some fish toward the net path, such fish would be herded into the section of the bridle unobscured by the mudcloud and likely escape beneath the lower bridle. We attempted to confirm this possibility by examining video observations from this area of the bridle to see if flatfish escaped or showed any herding behavior, but the results were equivocal because few fish, and probably only moving fish, were seen.

Third, we assumed that in changing the length of the bridles we did not alter trawl geometry in any way other than by increasing the area exposed to herding. As in the studies of Engås and Godø (1989a) and Ramm and Xiao (1995), increased bridle length in our study produced a small, but significant, reduction in wing spread (Table 1). If this change produced a change in footrope contact and net efficiency, then it would lead to a biased estimate of herding efficiency (net efficiency is explicitly assumed to be independent of bridle length in Eq. 4). Other possible changes in trawl geometry are that 1) at the longest length the upper bridle may sag sufficiently to touch the bottom within L_{off} and 2) at the shortest length the bridle tension may be sufficient to lift the wingtip off the bottom. With such changes, and perhaps even without, L_{off} may not be constant at all bridle lengths. Although we examined the performance of the trawl along the entire distance from the wing tips to the doors at the standard bridle length, we had insufficient ship-time to examine the performance at all bridle lengths to verify that the bridles performed as intended. We recommend that further studies of herding efficiency include additional research to verify that standard trawl performance is maintained for all experimental configurations. One approach for doing this is to simultaneously estimate escapement under the footrope, perhaps by using an auxiliary net attached beneath the trawl net as in Engås and Godø (1989b), so that the assumed independence of net efficiency and bridle length could be tested.

Fourth, we have conducted these experiments in relatively small areas that do not encompass all of the bottom conditions found over the areas covered by the bottom trawl surveys; therefore the results are potentially biased and certainly less variable than if they were conducted over such a range of conditions.

Bridle efficiency is only one component needed to determine trawl efficiency (i.e. the proportion of fish within the door path that are caught). The remaining component, for flatfish at least, is net efficiency (i.e. the proportion of fish within the net path that are caught). If estimates of net efficiency are also available, perhaps obtained with the use of an auxiliary net to capture escaping fish (Engås and Godø, 1989b), they can be included with estimates of bridle efficiency in Dickson's (1993a) model of the trawl fishing process to produce estimates of trawl efficiency. Such estimates could then be used to convert relative indices of fish abundance from trawl survey to absolute estimates of abundance.

Acknowledgments

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Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana*

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Abstract—The red snapper (*Lutjanus campechanus*) is currently under rigorous federal and state management in the Gulf of Mexico because of apparent overfishing. Management strategies implemented to promote recovery of the species are dependent upon knowledge of various demographic variables, including the ages of individuals, the distribution of these ages (cohort strength) within the population, and maximum longevity. Thus a dependable and precise aging method is of principal importance. Counts of annuli in otolith thin sections have been used to age many species of fish, including red snapper. However, the utility of this method for aging red snapper has been questioned by those who dispute both the apparent longevity (over 50 yr) of red snapper and the position of the first annulus within the red snapper otolith.

We counted annuli and assessed edge condition in sagittal otoliths of 3791 red snapper collected from the northern Gulf of Mexico off Louisiana during the periods from 1989 to 1992 and from 1995 to 1998. Opaque annuli were validated by marginal increment analysis to form once per year from December through June. Estimated ages ranged from 0.5 to 52.6 yr for individuals from 104 mm to 1039 mm total length and from 0.02 kg to 22.79 kg total weight. Among the 2546 specimens of known sex, both sexes evidenced rapid growth of individuals until about age 8–10 yr, after which growth slows considerably. Von Bertalanffy growth models for total length at age were significantly different for males and females, indicating differential growth between the sexes, with females typically obtaining larger sizes at older ages than do males.

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The red snapper, *Lutjanus campechanus* (Poey) (family: Lutjanidae), is resident on the continental shelves of the Gulf of Mexico (GOM) and northwest Atlantic Ocean from the Bay of Campeche, Mexico, to Massachusetts; however, it is found only occasionally north of Cape Hatteras, North Carolina (Rivas, 1966; Robins and Ray, 1986; Hoese and Moore, 1998). Although Rivas (1966) suggested that red snapper may also occur off Bermuda, the Bahamas, and northern Cuba, it has been reported neither from Bermuda by Smith-Vaniz et al. (1999) nor from the Bahamas by Böhlke and Chaplin (1993), nor from Cuba by Allen (1985). The species is replaced in the Caribbean Sea and southward by the Caribbean red snapper, *L. purpureus* (Rivas, 1966; Robins and Ray, 1986; Hoese and Moore, 1998). Although the appellation "red snapper" has been widely used to identify as many as 12 commercially marketed lutjanids (Camber, 1955; Carpenter, 1965), it is correctly applied only to *L. campechanus* (Robins et al., 1991). The binomina *L. aya, L. blackfordi,* and *Neomaenis aya*, all of which appear in the literature (e.g. Moseley, 1966) are synonyms of *L. campechanus* and refer to the red snapper *sensu* Robins et al. (1991).

The red snapper has been and remains a significant component of both the commercial and recreational fisheries in the GOM. However, documented commercial landings from United States territorial waters declined precipitously from historic highs of about 6389 metric tons (t) in 1965 to 1015 t in 1991; estimated recreational landings similarly waned from 4734 t in 1979 to 581 t in 1990 (Schirripa and Legault[1]). Since 1991 both fisheries have been constrained by size limits, creel or trip limits, and quotas as established by the Gulf of Mexico Fishery Management Council (GMFMC). The best efforts of the GMFMC and the commercial and recreational sectors notwithstanding, overfishing of red snapper in the GOM may persist (Schirripa and Legault[2]).

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* Contribution LSU-CFI-00-01 of the Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503.

[1] Schirripa, M. J., and C. M. Legault. 1997. Status of the red snapper in U. S. waters of the Gulf of Mexico: Updated through 1996. Contribution rep. MIA-97/98-05 from Sustainable Fisheries Division, Miami Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, 75 Virginia Beach Drive, Miami, FL 33149-1099. [Not available from NTIS.]

[2] Schirripa, M. J., and C. M. Legault. 1999. Status of the red snapper in U. S. waters of the Gulf of Mexico: Updated through 1998. Contribution rep. SFD-99/00-75 from Sustainable Fisheries Division, Miami Lab-

continued

Accurate information on the age structure of the red snapper populations in the GOM is essential for monitoring year-class strength, for conducting stock assessments, and for documenting population recovery. Previous efforts at estimating red snapper age have employed a variety of aging methods. Bradley and Bryan (1975) cited the long spawning season and a constant recruitment into the population as reasons for the difficulty in assigning red snapper ages from length-frequency data. Moseley (1966) used scale annuli to age red snapper to age 4 yr and advanced spawning as the causal factor in check formation. Among 240 red snapper taken off the west coast of Florida, Futch and Bruger (1976) estimated red snapper ages of 1 to 5 yr from 200 readable whole otoliths; however, they postulated ages up to 20 yr for larger individuals whose otoliths were unreadable. Comparisons of ages derived from whole otoliths, scales, and vertebrae by Bortone and Hollingsworth (1980) revealed all three hard parts to be of equal utility in aging red snapper at age 1 and 2 yr. Wade (1981) also used scales to age red snapper to 9 yr. Nelson and Manooch (1982) reported red snapper ages from 1 to 16 yr based on both scales and sectioned otoliths and demonstrated once yearly scale annulus formation in June and July from monthly mean marginal growth. A recent study of red snapper otoliths significantly extended the hypothesized longevity of red snapper in the GOM to 42 yr (Szedlmayer and Shipp, 1994). Render (1995) provided a preliminary validation of yearly opaque annulus formation in sagittal otoliths and reported ages from 0 to 53 yr for red snapper in Louisiana waters. Examinations of otolith sections from 537 red snapper captured in the northwestern Atlantic Ocean from Beaufort, North Carolina, south to the Florida Keys manifested a maximum longevity of 25 yr (Manooch and Potts, 1997). Among 907 red snapper from the GOM off Alabama, Patterson (1999) reported opaque annulus formation from January through June and maximum ages of 30 yr for females and 31 yr for males. Despite these efforts, the longevity of red snapper remains controversial. Small sample sizes, a paucity of older specimens, and the failure to present legitimate validations of ages from hard parts (Beamish and McFarlane, 1983) have variously hampered the above studies. It has further been speculated that larger and presumably older red snapper form numerous false annuli within otoliths (Rothschild et al.[3]). And both the timing of deposition and the position of the putative first annulus remain in question.

Otolith analyses have proven consistent in estimating ages of many fish species, including several from the temperate waters of the northern GOM (Johnson et al., 1983; Barger, 1985; Beckman et al., 1988; Beckman et al., 1990, 1991; Crabtree et al., 1992; Murphy and Taylor, 1994; Franks et al., 1998; Thompson et al., 1998). Herein we present our interpretations of the use of sagittal otoliths to estimate ages of red snapper from the GOM off Louisiana. Specifically we address the timing of formation and position of the first annulus, validation of the once yearly accretion of opaque annuli, longevity, and reader reproducibility. We further describe the growth of red snapper with von Bertalanffy growth models for both males and females.

Methods and materials

Red snapper from recreational and commercial catches were sampled from 1989 to 1992 and from 1995 to 1998 by personnel of the Louisiana State University (LSU) Coastal Fisheries Institute and the Louisiana Department of Wildlife and Fisheries (LDWF). Although the vast majority of our sampling efforts were targeted at both wholesale facilities and charter boat docks located in Grand Isle and Port Fourchon, LA, the area of coverage in the northern GOM extended from off the Mississippi River Delta in the east to off Galveston, TX, in the west. Morphometric measurements (total length [TL] or fork length [FL] in mm, total weight [TW] or eviscerated body weight [BW], i.e. mass with liver, digestive tract, and reproductive organs removed, in g) were recorded, both sagittal otoliths were removed, and sex was determined, when possible, for each specimen. Eviscerated body weight was converted to TW, when necessary, with the equation $TW=1.101(BW)-26.32$ (linear regression, df=418; $P<0.001$; $r^2=0.996$) and TL was estimated from FL with the equation $TL=1.073\ (FL)+3.56$ (linear regression, df=1015; $P<0.001$; $r^2=0.999$).

All undamaged, intact otoliths were weighed to the nearer 0.1 g. The left sagittal otolith from each individual was embedded in an epoxy resin and subsequently sectioned with a low-speed saw equipped with a wafering blade as described in Beckman et al. (1988). In those instances where the left sagitta was damaged or unavailable, the right sagitta was substituted. Examinations of otolith sections were made with a compound microscope and transmitted light at 40× to 100× magnification. Counts of annuli (opaque zones) were accomplished by reading along the medial surface of the transverse section dorsal or ventral to the sulcus; annuli were often inconsistent in other regions of the otolith section. Annuli were counted by two readers without knowledge of date of capture or morphometric data. The appearance of the otolith margin was also coded as either opaque or translucent (Beckman et al., 1988). Sections were recounted a second time by both readers when initial counts disagreed. Rather than excluding the small number of individuals for which a consensus could not be reached after a second reading (n=27), the assigned annulus count for these was that of the more experienced reader (reader 1). Reader 2's annulus count and edge condition were used in those circumstances where reader 1's were missing (n=2). Annulus counting error between the two readers was evaluated after both the initial and second readings of the otolith sections. Reproducibility of the resultant age estimates was evaluated with the coefficient of variation, the index of precision (Chang, 1982), and average percent error (Beamish and Fournier, 1981).

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[2] (continued) oratory, Southeast Fisheries Science Center, National Marine Fisheries Service, 75 Virginia Beach Drive, Miami, FL 33149-1099. [Not available from NTIS.]

[3] Rothschild, B. J., A. F. Sharov, and A. Y. Bobyrev. 1997. Red snapper stock assessment and management for the Gulf of Mexico. Report submitted to the National Marine Fisheries Service, Office of Science and Technology, 1315 East-West Highway, Silver Spring, MD 20910 USA. [Not available from NTIS.]

The periodicity of opaque annulus formation was determined by marginal increment analysis and by plotting the proportion of otoliths with opaque margins by month of capture (Beckman et al., 1988). To assess the possibility of false annulus formation among either younger or older red snapper, those individuals of age ≤5 yr and those >5 yr were also analyzed as above. Fork lengths at 100% maturity, 420 mm for males and 440 mm for females (Render, 1995), are achieved at about age 5. If one opaque and one translucent zone are shown to be formed each year, validation of annuli as being accreted once yearly is accomplished.

Ages of red snapper were estimated from opaque annulus counts and date of capture with the equation

$$Age(\text{days}) = -182 + (annulus\ count \times 365) + \left((m-1)\times 30\right) + d,$$

where m = the ordinal number (1–12) of the month of capture; and
d = the ordinal number (1–31) of the day of the month of capture.

The 182 days that were subtracted from each age estimate are an accommodation for the uniform 1 July hatching date which was assigned for all specimens (Render, 1995; Collins et al., 1996). Age in yr was derived by dividing the age in days by 365. Thus a red snapper captured on 1 January which exhibits five opaque annuli (including an opaque margin [see below]) in its otoliths would have an estimated age of 1644 days or 4.5 yr. Our age estimation method also assumed that opaque annulus formation at the otolith margin uniformly commenced in January. The small number of individuals captured in September, October, November, and December that evidenced early formation of opaque annuli had their ages adjusted by subtracting 365 days from their age estimates. Conversely, a larger number of individuals captured in January, February, and March had otoliths with translucent margins—evidence of an assumed delay in opaque annulus formation. The age estimates of these were augmented by the addition of 365 days.

Total length-TW regressions were fitted with linear regression to the model $TL = a\,TW^{b}$ from $\log^{10}$-transformed data. Male and female regressions were compared with analysis of covariance (SAS, 1985). Only those red snapper for which sex could be determined were used to fit growth models. Von Bertalanffy growth models of TL at age were fitted with nonlinear regression by least squares (SAS, 1985) in the form

$$TL_t = L_\infty\left(1 - e^{-k(t-t_0)}\right),$$

where TL_t = TL at age t;
L_∞ = the TL asymptote;
k = a growth coefficient;
t = age in yr; and
t_0 = a hypothetical age when TL is zero.

Growth models were generated for three groups of red snapper within which the age and TL of all individuals were extant: 1) all specimens, 2) all specimens of known sex, and 3) specimens of known sex for which growth models were fitted independently for each sex. Likelihood ratio tests (Cerrato, 1990) were used to test for differences between males and females, both in growth models and in growth parameter estimates. Significance level for statistical analyses was 0.05 unless indicated otherwise.

Results

During eight years of variable collection effort, 3791 red snapper from recreational (n=274) and commercial (n=3517) catches were sampled for morphometric data and sagittal otoliths. Among the 1438 male and 1542 female specimens for which sex could be determined, females ranged from 242 to 1039 mm TL and from 0.16 to 22.79 kg TW; males were 245–946 mm TL and 0.19–13.70 kg TW. Composite ranges for all specimens of either known or unknown sex were 104–1039 mm TL and 0.02–22.79 kg TW; however, 67.6% of 3787 available TL were between 325 and 525 mm and 80.0% of 3718 available TW were less then 2.5 kg (Fig. 1). Neither the slopes (df=2,932; F=3.41; P<0.065) nor the intercepts (df=2,932; F=3.16; P<0.075) of the TL-TW regressions were found to differ significantly between males and females; thus data for the two sexes were combined and a single predictive equation was generated

$$TW = 1.17 \times 10^{-8}\,(TL)^{3.04}$$

Sagittae of red snapper are ovate, laterally compressed, and have an indented sulcus acousticus on the proximal surface (Fig. 2A). Although one can count purported annuli in relatively small whole otoliths of red snapper less than age 5 (Futch and Bruger, 1976), it is difficult to discern annuli in the larger otoliths of older individuals. Thin transverse sections of these older otoliths showed semidistinct translucent and opaque annuli that alternated from the core to the growing edge (Fig. 2, B and C). The presumptive first annulus posed the most consistent problem for the readers. This annulus appeared as a diffuse "smudge" of opaque material variously located from totally isolated and somewhat distant from the core (Fig. 2B) to contiguous to and continuous with the otolith core (Fig. 2C).

Annulus counts ranging from 0 to 53 and edge conditions were determined by at least one reader for all 3791 individuals sampled. Reader 2 considered all the otolith sections to be of sufficient quality to produce annulus counts; reader 1 provided annulus counts from all but two sections. After the initial counts, consensus between readers was achieved for 2804 individuals A second reading of the 987 sections for which annulus counts differed produced consensus for 3762 individuals. The degree of agreement in red snapper opaque annulus counts between the two readers in each of the two readings was assessed. Average percent error (APE), coefficient of variation (CV), index of precision (D), and percentages of absolute differences in counts are given in Table 1.

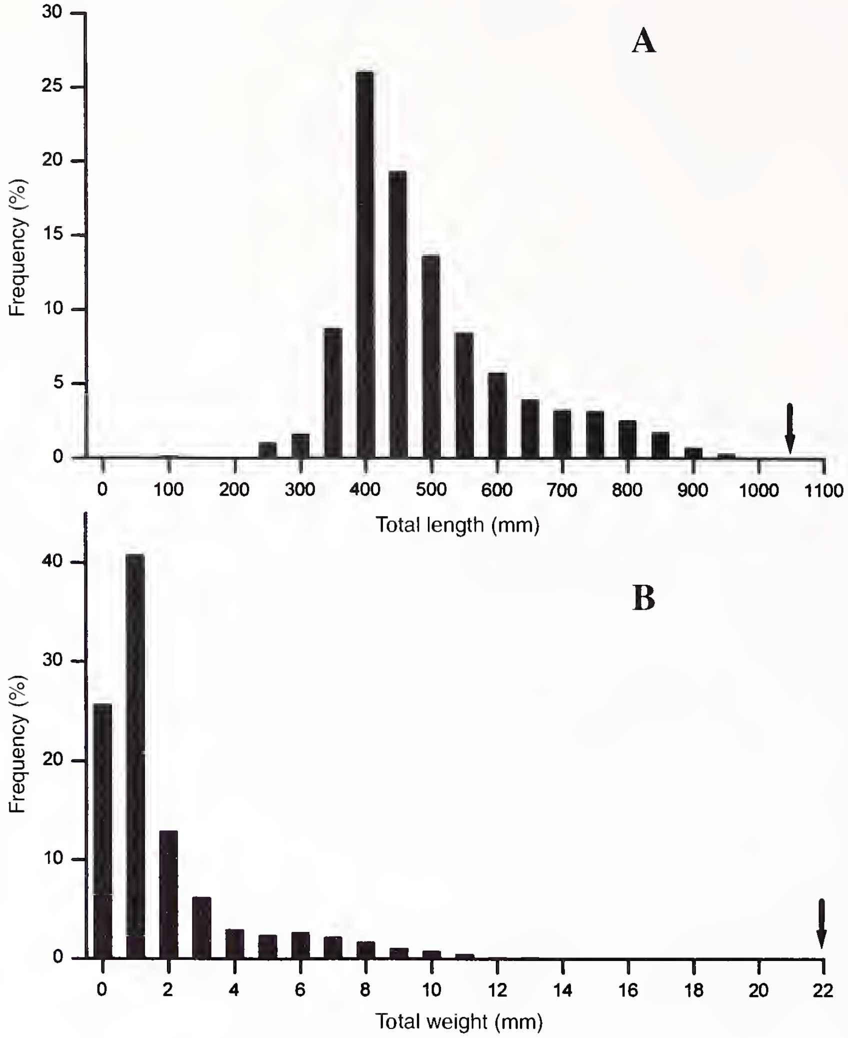

Figure 1

Frequency histograms for (**A**) total length in mm (n=3787) and (**B**) total weight in kg (n=3718) for red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. Specimens collected from the recreational and commercial fisheries from 1989 to 1992 and from 1995 to 1998. The vertical arrow denotes the rod and reel world record red snapper caught on 23 June 1996.

Proportions of otoliths with opaque margins were plotted by month of capture for all individuals (n=3791), for those individuals presumed to be sexually immature (ages less than or equal to 5, n=2143), and for those from individuals of presumptive sexual maturity (ages greater than 5, n=948). Each of the three plots (Fig. 3) features a single broad peak and a single broad valley and conclusively demonstrates opaque annulus formation from December through June and translucent annulus formation from July through November. Thus, the assumption of one to one correspondence between opaque annulus counts and estimated red snapper age in years is validated. Furthermore, this correspondence is validated for immature and mature individuals of all ages.

Having demonstrated once yearly accretion of opaque annuli, we estimated ages from 0.5 to 52.6 yr from the annulus counts of the red snapper in our study. The vast majority of specimens examined were ages 2–5 and only 1.2% of the total number were greater than age 15 yr (Fig. 4). The few ages greater than 15 yr, which were not represented in our sample, were 24, 28, 31, 34, 39, 40, 42–46, and 49–50. The otolith section from the oldest specimen examined is shown in Figure 5.

Among 3787 individuals, the single von Bertalanffy growth model which best describes red snapper TL at age was

$$TL\ (\text{mm}) = 941\ [1 - e^{-0.18\,(t\,+\,0.55)}], \qquad (r^2{=}0.72).$$

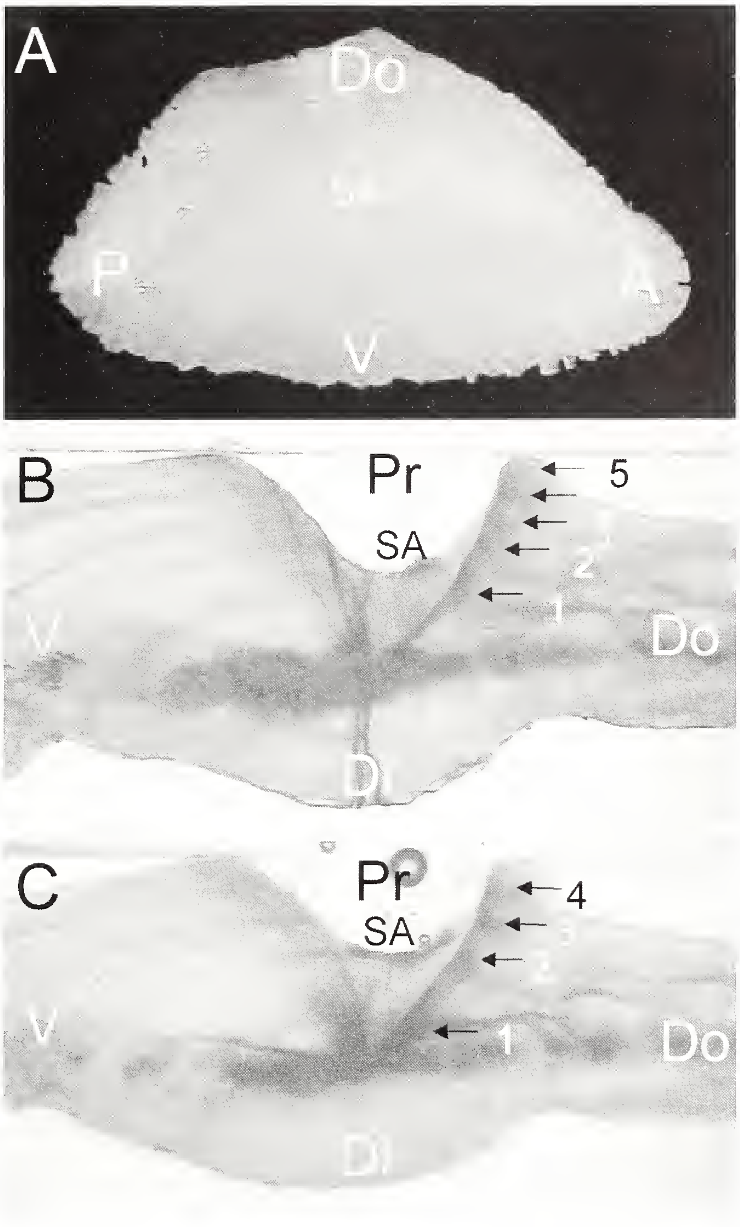

Figure 2

Medial view (**A**) and transverse sections (**B** and **C**) of red snapper (*Lutjanus campechanus*) left sagittal otoliths. Abbreviations for all are Do = dorsal, V = ventral, P = posterior, A = anterior, SA = sulcus acousticus, Di = distal, and Pr = proximal. Arrows with numbers indicate positions of opaque annuli and their enumeration.

Not surprisingly, the von Bertalanffy growth model generated for all individuals of known sex (n=2979) was quite similar to the above:

$$TL\ (\text{mm}) = 935\ [1 - e^{-0.18\,(t\,+\,0.54)}], \qquad (r^2=0.72).$$

However, a likelihood ratio test revealed that von Bertalanffy growth models for males (n=1438) and females (n=1541) were significantly different from one another (χ^2=75.09; df=1,2979; P<0.0001). The resultant models for TL at age were

$$Female\ TL\ (\text{mm}) = 977\ [1 - e^{-0.16\,(t\,+\,0.63)}], \qquad (r^2=0.71)$$
$$Male\ TL\ (\text{mm}) = 904\ [1 - e^{-0.19\,(t\,+\,0.48)}], \qquad (r^2=0.73)$$

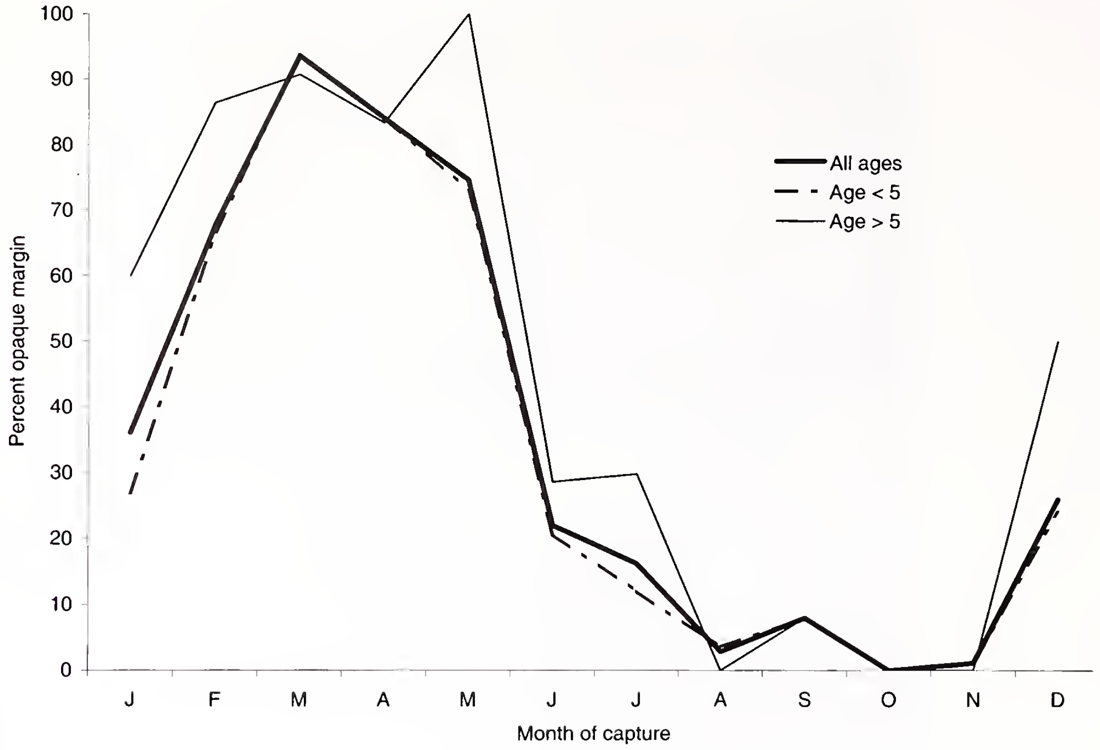

Figure 3

Marginal increment analysis of red snapper (*Lutjanus campechanus*) otoliths for specimens of all ages (n=3791), for ages >5 (n=2143), and for ages >5 (n=948).

Predicted TL at age for males and females (Figs. 6 and 7) generated with the above equations illustrated rapid and roughly equivalent growth to an age of approximately 8–10 yr after which the growth curves diverge. Differential growth between males and females was further demonstrated with likelihood ratio tests which indicated significant differences in L_∞ (χ^2=13.05; df=1,2979; P<0.0003) and k (χ^2=7.16; df=1,2979; P<0.0075). No significant difference was detected in t_0 (χ^2=1.34; df=1,2979; P<0.247). Owing to the large variability in observed TL at age (Fig. 6), TL was a poor estimator of red snapper age within the range of TL encountered in our sampling efforts.

Table 1

Average percent error (APE), coefficient of variation (CV), and index of precision (D) differences in red snapper (*Lutjanus campechanus*) otolith annulus counts for two readers on first and second readings. n = 3791.

	1st reading	2nd reading
APE	3.736	0.0934
CV	0.045	0.0011
D	0.032	0.0008
0	73.96%	99.29%
±1	23.27%	0.61%
±2	2.24%	0.08%
≥ ±3	0.53%	0.02%

Discussion

Otolith annuli as indicators of age in years have been validated for many freshwater and marine fish species, including the Australian lutjanids *L. adetii* and *L. quinquelineatus* (Newman et al., 1996) and *L. argentimaculatus*, *L. bohar*, *L.carponotatus*, *L. erythropterus*, *L. gibbus*, *L. johnii*, *L. malabaricus*, *L. monostigma, L. rivulatus*, *L. sebae*, and *L. vitta* (Cappo et al., 2000). Previous studies of red snapper have used scales (Moseley, 1966; Wade, 1981), otoliths (Futch and Bruger, 1976; Szedlmayer and Shipp, 1994; Render, 1995; Manooch and Potts, 1997; Patterson, 1999), scales and otoliths (Nelson and Manooch, 1982) and scales, otoliths, and vertebrae (Bortone and Hollingsworth, 1980) to estimate ages. Among these, early attempts to validate age estimation from circuli of scales and annuli of otoliths have suffered from two shortcomings: 1) a small sample size and 2) a paucity of individuals over age 10 yr. Nevertheless, they have produced a general consensus

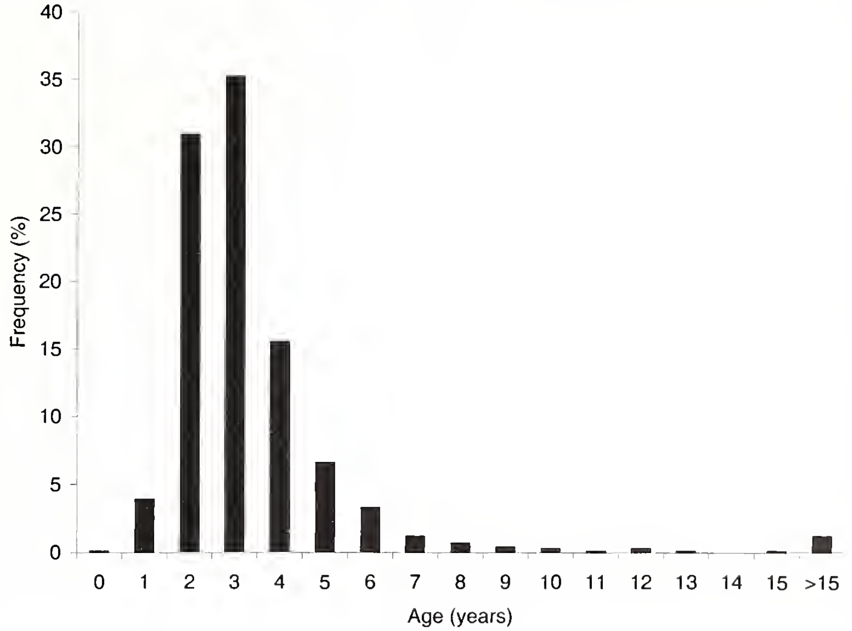

Figure 4

Age-frequency histogram (n=3791) for red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico. Specimens were collected from the recreational and commercial fisheries of the Gulf of Mexico off Louisiana from 1989 to 1992 and from 1995 to 1998.

that transparent annuli (Nelson and Manooch, 1982) are formed during the spawning season, May to September in the GOM (Collins et al., 1996). Our validation of opaque annulus formation in otoliths of red snapper during the winter and spring seasons is in substantial agreement with previous efforts. Given that yearly formation of opaque annuli has been validated for substantial numbers of red snapper from the Atlantic waters off North Carolina south to Florida (Manooch and Potts, 1997) and the GOM waters off Alabama (Patterson, 1999) and Louisiana (Render, 1995; our study), and the validation among Australian congeneric species cited above, the one-to-one correspondence between annuli and age in years should be indisputable.

Certainly, the reproducibility statistics indicate that the annuli of red snapper otoliths are more difficult to count than those in otoliths of some other species. Comparisons of between-reader age estimates in several species of the family Sciaenidae have yielded almost 100% agreement (Beckman et al., 1988; Beckman et al., 1990; Barbieri et al., 1993; Lowerre-Barbieri et al., 1995). Sciaenid otoliths are comparatively massive and annuli are especially well defined. Conversely, red snapper otoliths are relatively thin and fragile and the annuli become less well defined with increasing age. But, even given the above, a first reading followed by a second reading produced consensus in age estimates for 99.29% of those red snapper considered in our study. Patterson (1999) reported 93.8% between-reader consensus of red snapper annulus counts after two readings. Quite unlike the situation in sciaenids, training and experience are critical to achieving high between-reader consensus on red snapper annulus counts.

The variable position and the diffuse appearance of the first annulus formed during the first winter following hatching (age approximately 6 months) are presumed to be functions of both the protracted red snapper spawning season and the rapid growth rate of juvenile red snapper. Those individuals that are spawned early in the season will experience proportionally more growth (and presumably more translucent zone accretion adjacent to the otolith core) than will a late spawned individual before opaque annulus accretion begins during the following winter; thus the first opaque annulus will be more distant from the otolith core in the former instance than under the latter circumstance. Also with the first opaque annulus accreting at a rate theoretically corresponding to the rapid growth rate experienced during the juvenile stage, the resulting first annulus is broader and more diffuse in appearance than annuli produced during times of reduced growth rates in later life. A more complete understanding of first annulus formation in red snapper could improve insights into both recruitment patterns and growth rates of individuals within a spawning season.

The distribution of ages among our sample population (Fig. 4) is certainly not reflective of the age distribution of red snapper in the GOM off Louisiana. Age-0 and age-1 specimens have been largely unavailable to our sampling efforts owing to minimum size limits applied to the recreational and commercial fisheries during the 1990s. Also

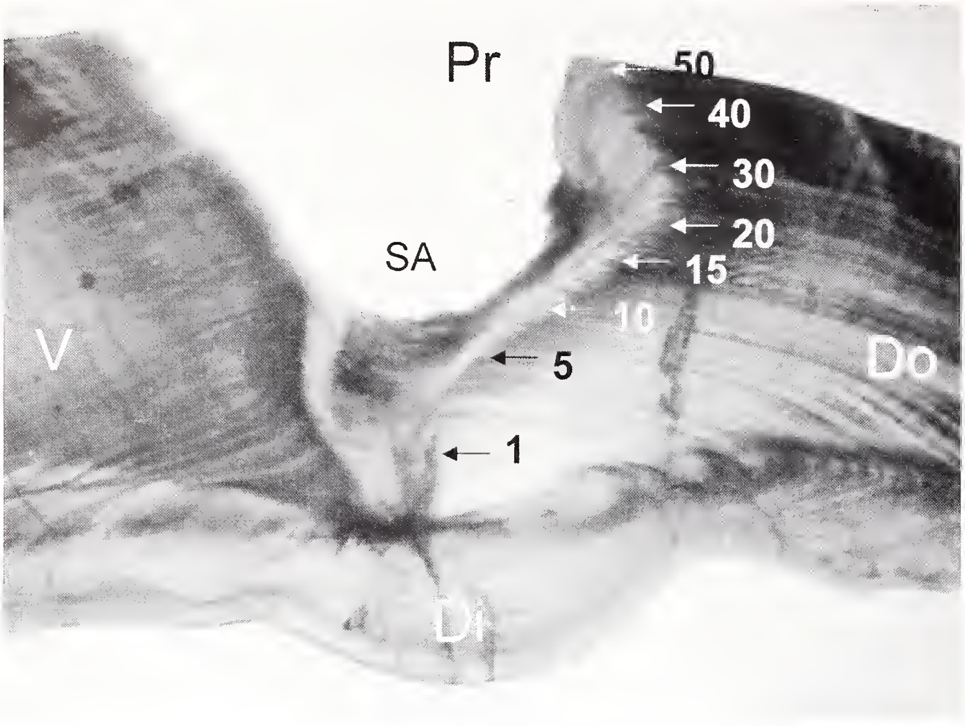

Figure 5

Cross section of sagittal otolith from the oldest red snapper, *Lutjanus campechanus,* from among 3791 specimens sampled from the recreational and commercial fisheries of the Gulf of Mexico off Louisiana from 1989 to 1992 and from 1995 to 1998. Abbreviations are Do = dorsal, V = ventral, SA = sulcus acousticus, Di = distal, and Pr = proximal. Arrows with numbers indicate positions of opaque annuli and their enumeration.

the dominance of ages 2–5 may reflect the practices of the fishermen who target red snapper and a migratory aspect of the species' life history. Age-0, and to a lesser extent age-1, red snapper are known to inhabit shallow water areas devoid of complex habitats or vertical relief where some are vulnerable to capture in trawls. This behavior is illustrated in fishery-independent trawl data from the GOM, specifically the Fall Groundfish Survey and the Summer SEAMAP Survey, in which the great majority of red snapper captured are age 0 and 1 (Schirripa and Legault[2]). It has been hypothesized that the disappearance of red snapper from the trawl data at age 1 represents their migration to structures such as oil and gas platforms that presumably provide refuge from large predators (Render, 1995). It is during this residence at the numerous oil and gas platforms off Louisiana that red snapper become vulnerable to fishing gear. Because the platforms are easily located and potentially harbor large populations of red snapper and other fish species (Stanley and Wilson, 1996, 1998), they are the preferred destinations for both commercial and recreational fishermen. The very low numbers of individuals of age >6 in our sample population likely result from both removal from the population through fishing and natural mortalities and emigration away from the oil and gas platforms to alternative habitats where they are less susceptible to capture.

It is difficult to compare the maximum observed red snapper longevity reported in our study with those reported in earlier studies (Moseley, 1966; Futch and Bruger, 1976; Wade, 1981; Nelson and Manooch, 1982; Render, 1995; Szedlmayer and Shipp, 1994; Patterson, 1999) because of the assortment of aging techniques (scales, whole or sectioned otoliths, length frequencies) and the variety of sources (commercial, recreational, or both) used. All show a predominance of relatively young individuals (<10 yr). However, recent advances and refinements in otolith preparation technology have allowed red snapper to be aged reliably up to the following ages: 42 yr (Szedlmayer and Shipp, 1994), 53 yr (Render, 1995), 31 yr (Patterson, 1999), and 52 yr (our study). Despite the sparsity of old red snapper among these research efforts, there can be little doubt that red snapper at least have the potential to achieve ages of 40–50 yr and more.

The red snapper growth models that we present are similar to those of earlier studies (Nelson and Manooch, 1982; Szedlmayer and Shipp, 1994; Manooch and Potts, 1997; Patterson, 1999) which did not produce separate models for the two sexes and variously applied weighted

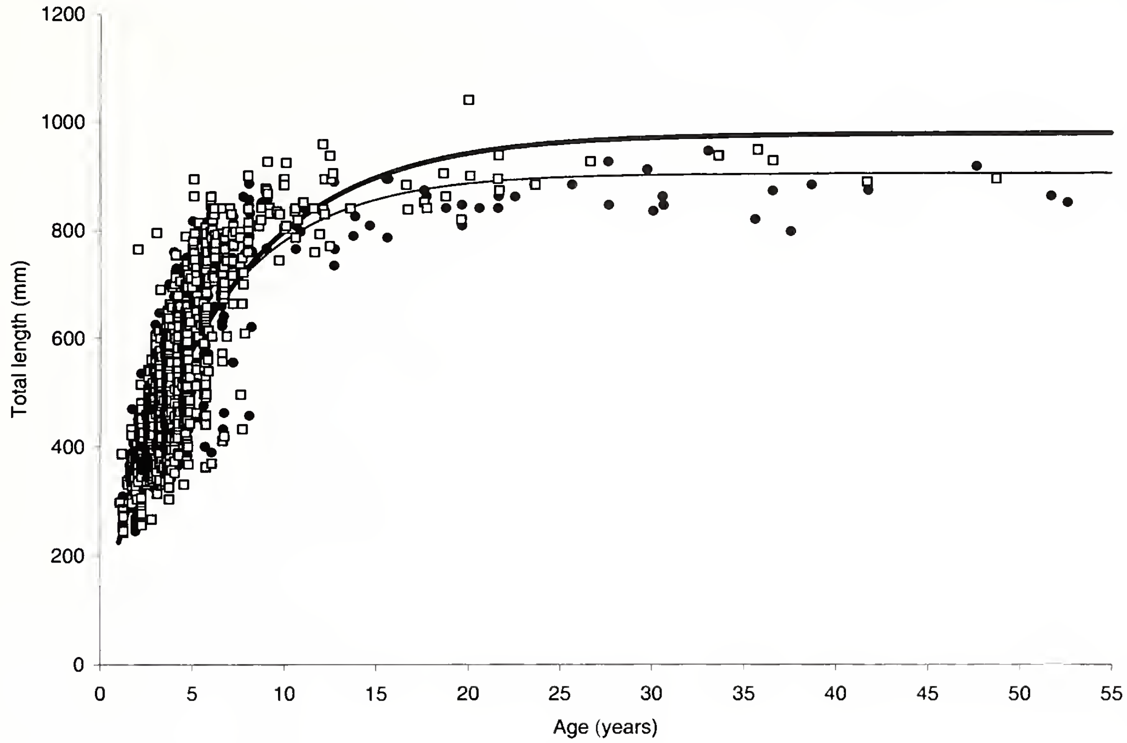

Figure 6

Observed total length (mm) at age and relationship of age to total length predicted from von Bertalanffy growth models for male (n=1438) and female (n=1542) red snapper, *Lutjanus campechanus,* from the northern Gulf of Mexico. Closed circles and narrow line represent males; open squares and thick line represent females.

or unweighted analyses (Fig. 7). All models predict rapid, and very much similar, growth during the first 8–10 years of life and slower growth thereafter. Asymptotic lengths among the above varied from 936 to 1025 mm. Our von Bertalanffy growth models also predicted a greater asymptotic TL and slightly faster growth for female red snapper. Among marine teleosts from the GOM, a similar pattern of growth has been shown for red drum, *Sciaenops ocellatus* (Beckman et al., 1988), sheepshead, *Archosargus probatocephalus* (Beckman et al., 1991), and cobia, *Rachycentron canadum* (Franks et al., 1998). However, this phenomenon in red snapper may be the result of a preponderance of data from the commercial fishery included in our analyses. Owing to minimum size limits enforced in both the commercial and recreational fisheries, we had access to few red snapper less than age 2 and few less than 250 mm TL. Conversely, the preference of commercial red snapper fishermen and wholesalers for smaller, plate-size individuals afforded us little opportunity to sample larger, and presumably older, red snapper; these are the individuals that can influence estimation of k and which ultimately drive estimation of L_∞. The addition of another 20–30 old specimens of both genders could have profound effects on the estimations of L_∞ for both sexes. Furthermore, growth studies of lutjanids in Australian waters (Davis and West, 1992; McPherson and Squire, 1992; Newman et al., 1996) report faster growth and larger size at age among males. Proportionally greater expenditures of energy in the production of gametes by females is advanced to explain this observation (Newman et al., 1996). Thus, although the growth rates and asymptotic lengths for male and female red snapper are shown to differ statistically in our study, questions of the biological veracity and the biological significance of these differences remain unresolved.

In addition to the differences in red snapper estimated growth rates between the sexes, there is an obvious high degree of diversity in individual growth rates. Owing to the large variability in age at a given TL (Fig. 6), this variable is a poor estimator of red snapper age. Our data indicate that red snappers of 400 mm, 600 mm, and 800 mm TL could be ages 2–7 yr, 3–9 yr, and 5–35+ yr, respectively. As a more concrete example, consider the International Game Fishing Association world-record red snapper caught by rod and reel, the otoliths of which were given to us for age analysis. This individual was caught off the coast of Louisiana by Doc Kennedy of Grand Isle, LA, on 23 June 1996; it was 22.79 kg (50 lb, 4 oz) TW, 1039 mm (40.9 in) TL, and 965 mm (38 in) FL. Given the immense size of this specimen, one would reasonably expect it to be ancient by red snapper standards. However, our analysis

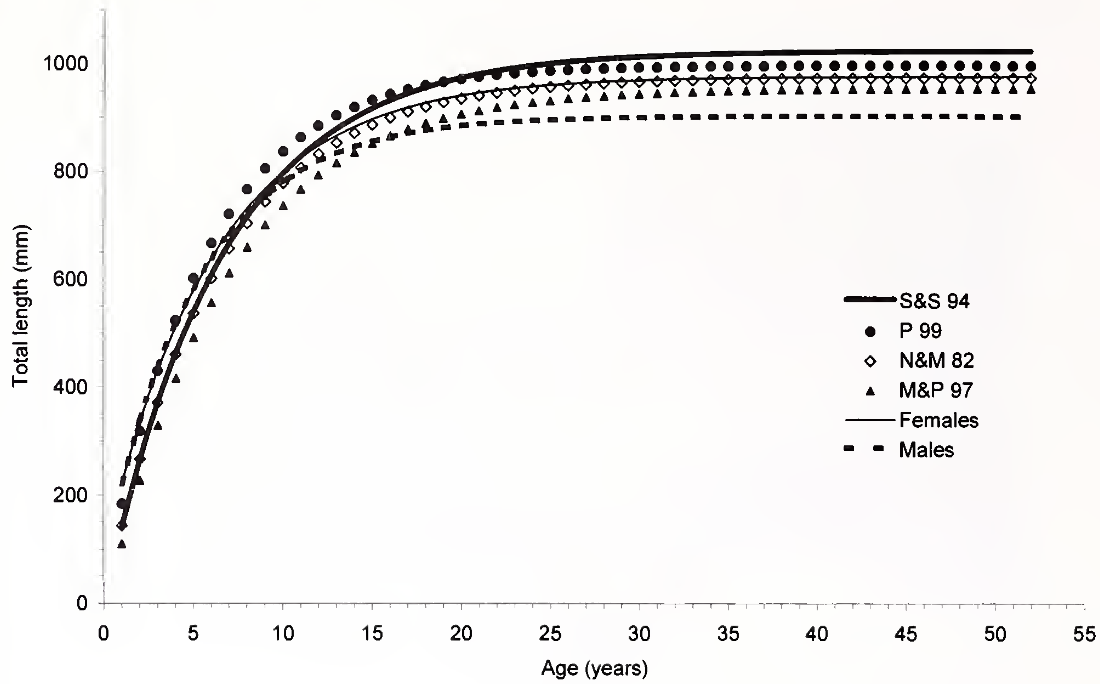

Figure 7

Comparative von Bertalanffy growth models for red snapper, *Lutjanus campechanus*, from the western north Atlantic Ocean. S&S 94 = Szedlmayer and Shipp (1994), P 99 = Patterson (1999), N&M 82 = Nelson and Manooch (1982), and M&P 97 = Manooch and Potts (1997). Males and Females are from our study.

revealed it to be only 19.98 yr. Conversely, the two oldest red snapper we encountered, age 52.63 and 51.73 yr, were a comparatively small 851 mm TL and 862 mm TL, respectively, and 7.886 kg TW and 9.188 kg TW, respectively. A similar pattern was noted by Patterson (1999) among the red snapper that he sampled from the GOM off Alabama.

Personnel at the LSU Coastal Fisheries Institute continue to investigate the nuances of deriving red snapper ages from sagittal otoliths. Although our marginal increment analysis demonstrates that a single opaque increment is formed each year, our sample size among older individuals, albeit larger than any previous investigation, is probably inadequate for absolute validation of this phenomenon. Thus, some have and will continue to question once yearly annulus accretion among red snapper older than 20 yr. A solution for this problem may lie in radiometric aging techniques with protocols that analyze various radionuclides in the otoliths. Also, core-to-first-annulus measurements made on otolith sections from age-0 and age-1 individuals would contribute to a better understanding of when and how the first annulus is accreted.

Acknowledgments

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Stomach content analysis of cobia, *Rachycentron canadum*, from lower Chesapeake Bay*

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Cobia (*Rachycentron canadum*) is a migratory pelagic species that is found in tropical and subtropical seas of the world, except in the central and eastern Pacific Ocean. The United States ranks third in total commercial production of cobia, however, recreational landings generally exceed commercial landings by an order of magnitude (Shaffer and Nakamura, 1989). In the western Atlantic Ocean, cobia migrate to Chesapeake Bay in spring and summer to spawn, and the productive waters of the Bay are believed to constitute important foraging grounds (Joseph et al., 1964; Richards, 1967). Cobia are known to move to areas of high food abundance, particularly crustacean abundance (Darracott, 1977).

Recent feeding studies in the northern Gulf of Mexico and off North Carolina have reported geographic differences in cobia diet and have indicated that the relative importance of fishes versus crustaceans is variable and that cephalopods constitute the least significant prey items. In the northern Gulf of Mexico, Franks et al. (1996) reported that fish (primarily anchovies, *Anchoa* sp.) dominated (% index of relative importance [IRI], Pinkas et al., 1971) the diet of juvenile cobia (236–440 mm FL). Meyer and Franks (1996) reported crustaceans (primarily portunid crabs) occurred in 79.1% of stomachs and represented 77.6% of total prey items consumed by cobia (373–1,530 mm FL) in the northern Gulf of Mexico. Fish (primarily hardhead catfish, *Arius felis*, and American eel, *Anguilla rostrata*) increased in importance with increasing cobia size. Fish were found in 58.5% of all cobia stomachs (20.3% of total prey) but occurred in 84.4% of stomachs of cobia 1150–1530 mm FL (Meyer and Franks, 1996). In contrast, Smith (1995) observed decreased importance of teleosts in the diet of cobia (39–142 cm FL) in North Carolina. Elasmobranch fishes and portunid crabs dominated the diet of cobia >9 kg (Smith, 1995).

Tag-recapture data collected between 1995 and 1999 document localized movement of cobia within lower Chesapeake Bay during summer, as well as the return of individual cobia to specific locations or general regions of the lower Bay in subsequent summers.[1] Although Chesapeake Bay is an important destination for migrating cobia, feeding habits of cobia in the Bay have never been thoroughly examined. Our study documents cobia feeding habits in Chesapeake Bay and compares findings with similar cobia studies from North Carolina and the northern Gulf of Mexico.

Methods

Cobia were sampled opportunistically at marinas and fishing tournaments in lower Chesapeake Bay between June and July 1997. Intact stomachs were removed by cutting above the cardiac sphincter (esophagus) and below the pyloric sphincter (large intestine). Stomachs were labeled, bagged, transported on ice to the VA Institute of Marine Science, and examined in relatively fresh condition. An incision was made along the longitudinal axis and the contents of stomachs were emptied onto a 500-µm mesh sieve for rinsing and sorting. Contents were blotted dry on paper towels before counts, displaced volumes (1-L graduated cylinder), and identifications to the lowest possible taxon were made. When possible, carapace widths (mm) of crabs were measured with calipers. An index of relative importance (IRI) for all prey items combined was calculated with the formula (*% Number* + *% Volume*) × (*% Frequency*), as described by Pinkas et al. (1971) and subsequently used by Smith (1995), Meyer and Franks

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* Contribution 2390 of the Virginia Institute of Marine Science, Gloucester Point, VA 23062.

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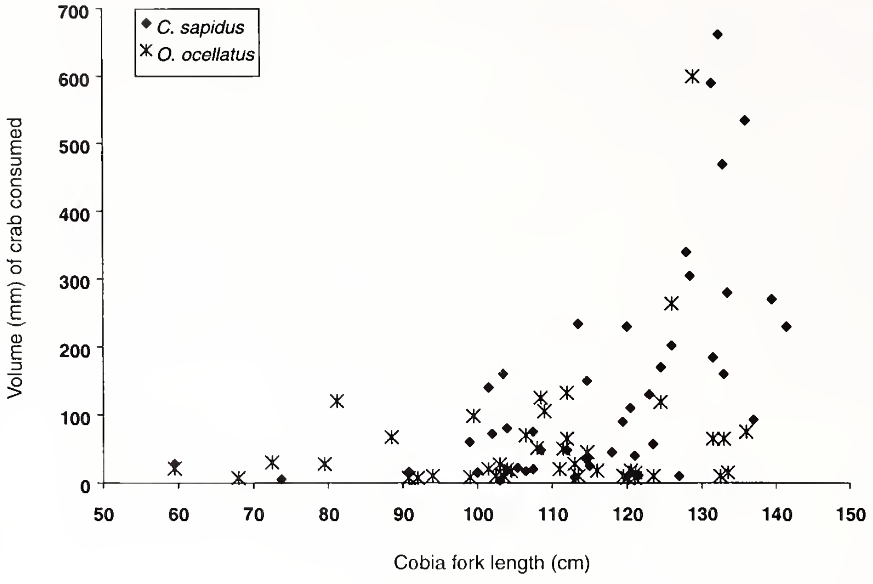

Figure 1

Volume (mL) of portunid crab consumed in lower Chesapeake Bay as a function of cobia size.

(1996), and Franks et al. (1996). Frequency (%*F*) was the percent of all stomachs that contained food.

Results and discussion

Stomach contents from 114 adult cobia (37–141 cm FL) were examined. Seventy-eight stomachs (68%) contained at least one identifiable, nonbait prey item. One species of bivalve, one species of hydroid, six species of crustacean, one elasmobranch, and 16 species of teleost were observed (Table 1). Mean volume of prey per stomach was 150.6 mL (range: 2–680 mL). Mean number of different prey per stomach was 1.9 species (range, 1–5 species). Blue crab (*Callinectes sapidus*) and lady crab (*Ovalipes ocellatus*) dominated the diet of cobia.

Index of relative importance (IRI) values for blue crab (5257) and lady crab (3665) were two orders of magnitude higher than IRI values for other prey items. Larger cobia consumed greater volumes of crab (Fig. 1). Additionally, larger cobia consumed larger (sublegal, <13 cm) blue crabs, although similar-size lady crabs were consumed by all sizes of cobia (Table 2). Atlantic croaker (*Micropogonias undulatus*), hogchoker (*Trinectus maculatus*), and fish and crab remains constituted the top food items volumetrically after portunid crabs. Although relatively high volumes were observed, IRI values for Atlantic croaker and hogchoker were low owing to infrequent occurrence of these items. High volume of crab remains was consistent with high volumes, counts, and frequency of occurrence of portunid crabs in cobia stomachs. Fish remains likely resulted from finfish bait (predominantly menhaden, *Brevoortia tyrannus*, and Atlantic croaker, *Micropogonias undulatus*). Crab and fish remains not identifiable to family and finfish bait were excluded from IRI calculations.

Consumption of blue crab was greatest along the western shore of the Bay and least at the mouth of the Bay. Conversely, consumption of lady crab was greatest at the mouth of the Bay and least along the eastern shore of the Bay (Fig. 2A). A greater percentage of lady crabs were consumed by male cobia than by female cobia, whereas a greater percentage of blue crabs were consumed by female cobia (Fig. 2B). IRIs for blue crab and lady crab were similar in June, but dramatically different in July. In July, IRI for blue crab was twice as high as that for lady crab. Prey availability and habitat utilization were likely responsible for determining location, sex, and within-season differences in composition of portunid crabs consumed by cobia. June samples were predominantly male cobia collected from the Bay mouth and eastern shore of the Bay, whereas July samples were predominantly female cobia collected from the western Bay shore.

Cobia feeding habits in lower Chesapeake Bay were more similar to feeding habits reported for cobia from North Carolina (Smith, 1995) than to feeding habits of cobia from the northern Gulf of Mexico (Franks et al., 1996; Meyer and Franks, 1996). Portunid crabs dominated the diet of cobia in Chesapeake Bay and North Carolina (Smith, 1995). Elasmobranch fishes were consumed exclusively by large cobia in Chesapeake Bay and North Carolina (Smith, 1995). In North Carolina (Smith, 1995), cobia fed on stingrays (*Dasyatis* sp.) and smooth dogfish (*Mustelus canis*). Cownose ray (*Rhinoptera bonasus*), previously unreported as a food item of cobia, was the only elasmobranch consumed by cobia in Chesapeake Bay. Cownose ray was observed from eight cobia stomachs; however, on-

Table 1

Index of relative importance (IRI) of prey items consumed by cobia (n=78) in lower Chesapeake Bay, June–July 1997. IRI was calculated as *(% Number + % Volume)* × *(% Frequency)*, as described in Smith (1995) and Meyer and Franks (1996). Frequency (*%F*) is the percent of stomachs that contained food. UnID = unidentified.

	Frequency (stomachs)	*%F*	Number (counts)	*%N*	Volume (mL)	*%V*	IRI
Phylum Cnidaria							
Class Hydrozoa							
Family Sertulariidae							
Sertularia sp.	1	1.28	1	0.14	8	0.07	0
Phylum Mollusca							
Class Bivalva							
Family Mytilidea							
Mytilus edulis	2	2.56	47	6.60	17	0.14	17
Phylum Arthropoda							
Class Crustacea							
Family Squillidae							
Squilla empusa	2	2.56	2	0.28	11	0.09	1
Family Pagruidae	3	3.85	3	0.42	5	0.04	2
Family Portunidae							
Callinectes sapidus	46	58.97	226	31.74	6736	57.31	5252
Ovalipes ocellatus	43	55.13	321	45.08	2505	21.31	3660
Family Canceridae							
Cancer sp.	2	2.56	2	0.28	26	0.22	1
Cancer irroratus	1	1.28	1	0.14	5	0.04	0
Family Xanthidae							
Neopanope sp.	1	1.28	3	0.42	5	0.04	1
Phylum Chordata							
Class Chonrichthyes							
Family Myliobatidae							
Rhinoptera bonasus	7	8.97	8	1.12	81	0.69	16
Class Osteichthyes							
Family Clupeidae							
Opisthonema oglinum	1	1.28	3	0.42	160	1.36	2
Brevoortia tyrannus	1	1.28	1	0.14	180	1.53	2
UnID Clupeidae	2	2.56	2	0.28	275	2.34	7
Family Batrachoididae							
Opsanus tau	1	1.28	1	0.14	185	1.57	2
Family Ophidiidae							
Ophidion marginatum	1	1.28	1	0.14	42	0.36	1
Ophidion sp.	3	3.85	4	0.56	47	0.40	4
Family Sygnathidae							
Syngnathus floridae	1	1.28	1	0.14	2	0.02	0
Syngnathus sp.	2	2.56	2	0.28	9	0.08	1
Hippocampus erectus	1	1.28	1	0.14	3	0.03	0
Hippocampus sp.	5	6.41	49	6.88	52	0.44	47
Family Pomatomidae							
Pomatomus saltatrix	1	1.28	1	0.14	180	1.53	2
Family Sciaenidae							
Micropogonias undulatus	3	3.85	3	0.42	663	5.64	23
Family Uranoscopidae							

continued

Table 1 (continued)

	Frequency (stomachs)	%F	Number (counts)	%N	Volume (mL)	%V	IRI
Family Uranoscopidae (continued)							
Astroscopus sp.	1	1.28	1	0.14	40	0.34	1
UnID Pleuronectiformes	3	3.85	4	0.56	5	0.04	2
Family Bothidae							
Paralichthyes dentatus	2	2.56	2	0.28	25	0.21	1
Scopthalamus aquosus	1	1.28	1	0.14	15	0.13	0
UnID Bothidae	1	1.28	1	0.14	2	0.02	0
Family Soleidae							
Trinectes maculatus	9	11.54	14	1.97	440	3.74	66
UnID Soleidae	3	3.85	3	0.42	15	0.13	2
Family Diodontidae							
Chilomycterus schoepfi	3	3.85	3	0.42	15	0.13	2
Totals			712				11754

Table 2

Consumption of portunid crabs as a function of five cobia size classes in lower Chesapeake Bay.

	Cobia size class (cm FL)				
	<100	100–109.9	110–119.9	120–129.9	>130
Callinectes sapidus					
n	9	16	12	23	42
Mean carapace width (mm)	55.2	72.3	75.0	116.7	94.7
Range of carapace width (mm)	33–86	42–120	45–120	42–120	35–120
Ovalipes ocellatus					
n	42	37	38	32	27
Mean carapace width (mm)	39.1	41.2	37.7	45.3	37.1
Range of carapace width (mm)	26–50	30–56	20–51	20–51	27–55

ly the jaw plates (22–49 mm width) remained; thus, the IRI for cownose ray is likely underestimated. Flatfishes and syngnathids represented the teleosts most frequently consumed by cobia in Chesapeake Bay and North Carolina. Hogchoker (*Trinectes maculatus*) was more frequently consumed by cobia in Chesapeake Bay (our study) than in North Carolina (Smith, 1995). Blackcheek tonguefish (*Symphurus plaigusa*) was regularly consumed by cobia in North Carolina (Smith, 1995) but was absent from cobia stomachs in Chesapeake Bay. In Chesapeake Bay, *Syngnathus* sp. (pipefish) and *Hippocampus* sp. (seahorse) were only important in the diet of smaller cobia, whereas in North Carolina, these fishes were important in the diet of all cobia (Smith, 1995).

Cobia in our study predominantly consumed benthic and epibenthic prey items, most notably portunid crabs. Feeding studies on other large predatory fishes in Chesapeake Bay during the summer months, such as bluefish, *Pomatomous saltatrix*, and weakfish, *Cynoscion regalis*, reveal that these species consume prey items associated predominantly with pelagic food webs (Hartman and Brandt, 1995a, 1995b). Striped bass, *Morone saxatalis*, a large predator present in Chesapeake Bay year-round, is also reported to feed predominantly on pelagic fishes (Hartman and Brandt, 1995a, 1995b; Walters, 1999). During the summer, portunid crabs were consumed by bluefish, weakfish, and striped bass in Chesapeake Bay; however, portunid crab consumption was typically less than 5% of total prey items present in the stomachs of these fishes (Hartman and Brandt, 1995a, 1995b; Walters, 1999). Red drum, *Sciaenops ocellatus,* a large predator that uses Chesapeake Bay between spring and fall is also reported to selectively consume portunid crabs in estuarine environments in the Gulf of Mexico (Scharf and Schlict, 2000); however, feeding habits of red drum in Chesapeake Bay have not been documented. Although

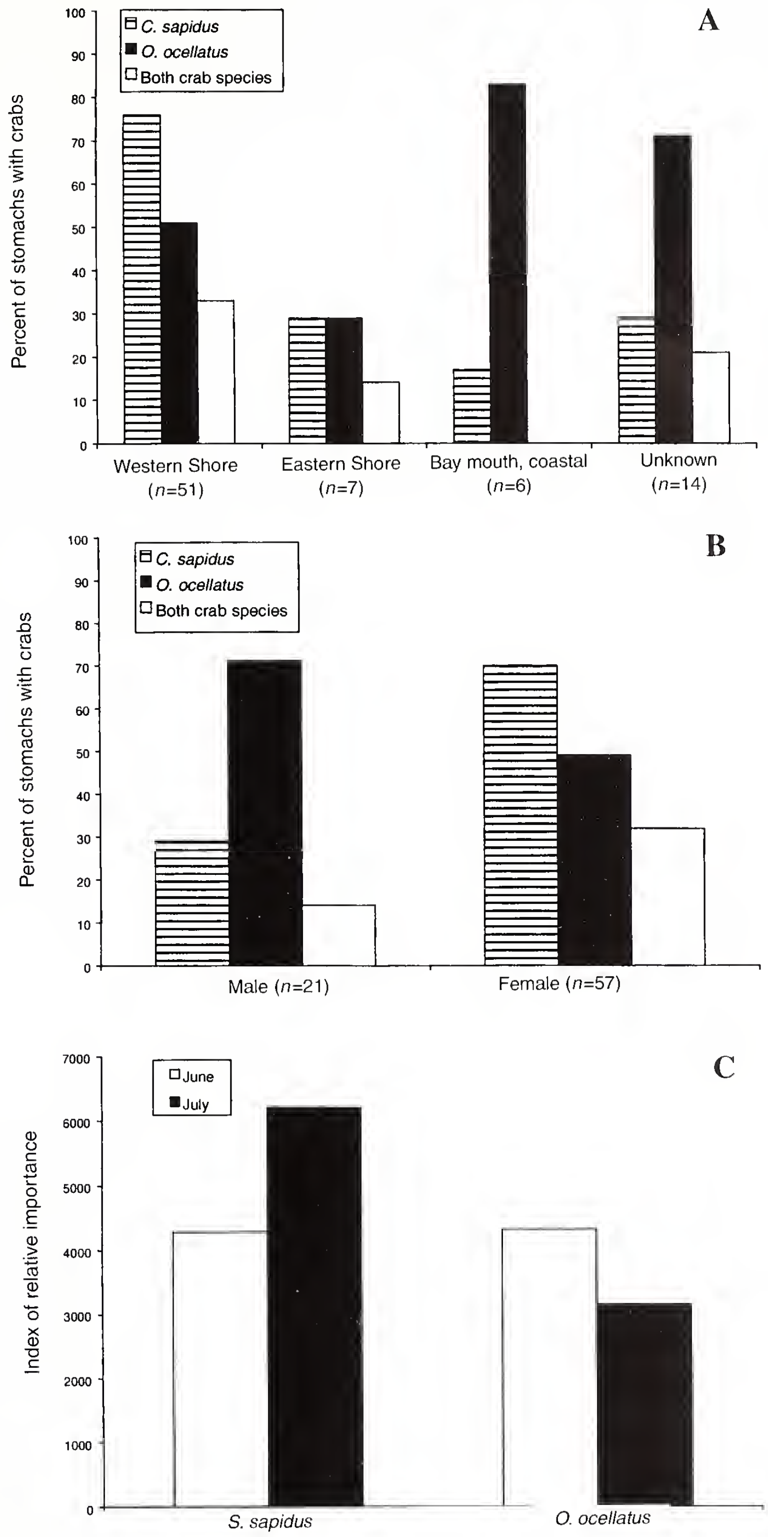

Figure 2

Consumption of blue crab (*Callinectes sapidus*) and lady crab (*Ovalipes ocellatus*) by cobia in lower Chesapeake Bay by location (**A**), sex (**B**), and month (June and July, **C**).

other large predators in Chesapeake Bay occasionally consume portunid crabs, cobia is the only large predator in Chesapeake Bay for which selective consumption of portunid crabs is documented.

Acknowledgments

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Preliminary genetic population structure of southern flounder, *Paralichthys lethostigma*, along the Atlantic Coast and Gulf of Mexico

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Southern flounder, *Paralichthys lethostigma*, inhabit coastal waters from Albemarle Sound, North Carolina to the Baja Laguna Madre del Sur in northern Mexico, but they are apparently absent from southern Florida (Ginsburg, 1952). This species inhabits coastal bays, sounds, and lagoons from spring to fall and migrates offshore to spawn in late fall and winter (Stokes, 1977). Valuable sport and commercial fisheries for southern flounder exist in both the northern Gulf of Mexico (Warren et al.[1]; Robinson et al.[2]) and the western North Atlantic (Monaghan[3]).

Declines in southern flounder absolute abundance in some regions (e.g. Texas during the 1980s; Fuls and McEachron[4]) have prompted some management agencies to institute restrictions on recreational and commercial fisheries including reductions in bag limits and minimum size. Should these measures fail to recover this fishery, other measures may be considered by managers, including further restrictions on harvest, or artificial propagation and stocking, or both. Implementation of such enhancement programs requires that genetic surveys be conducted to determine genetic variability and stock structure of managed fish populations (King et al., 1995). Failure to understand underlying genetic structure prior to implementing stocking programs places the genetic resources of target species at risk (Allendorf et al., 1987) and may result in the reduction or loss of among-population variability and changes in within-population genetic characteristics. Genetic analyses of population structure may also provide insight into management options that do not require stocking (Nelson and Soulé, 1987).

The objective of our study was to characterize population structure of southern flounder in coastal regions of the northern Gulf of Mexico and northwestern Atlantic Ocean and to test the null hypothesis of no genetic differentiation within the region surveyed. If this hypothesis was rejected, a number of processes would operate to structure southern flounder population(s). Genetic differentiation in some nearshore organisms in the northern Gulf of Mexico (e.g. *Sciaenops ocellatus*; Gold and Richardson, 1999) has been explained as isolation by distance (Wright, 1943). This model describes a population structured by isolation caused by limited individual migration potential in relation to the size of the species' distribution (Kimura and Weis, 1964). The hypothesis of isolation by distance is supported when geographic distance and genetic distance are positively correlated. Alternatively, differentiation may arise as an adaptive response to localized environmental conditions (King and Zimmerman, 1993) or from the operation of physical barriers, such

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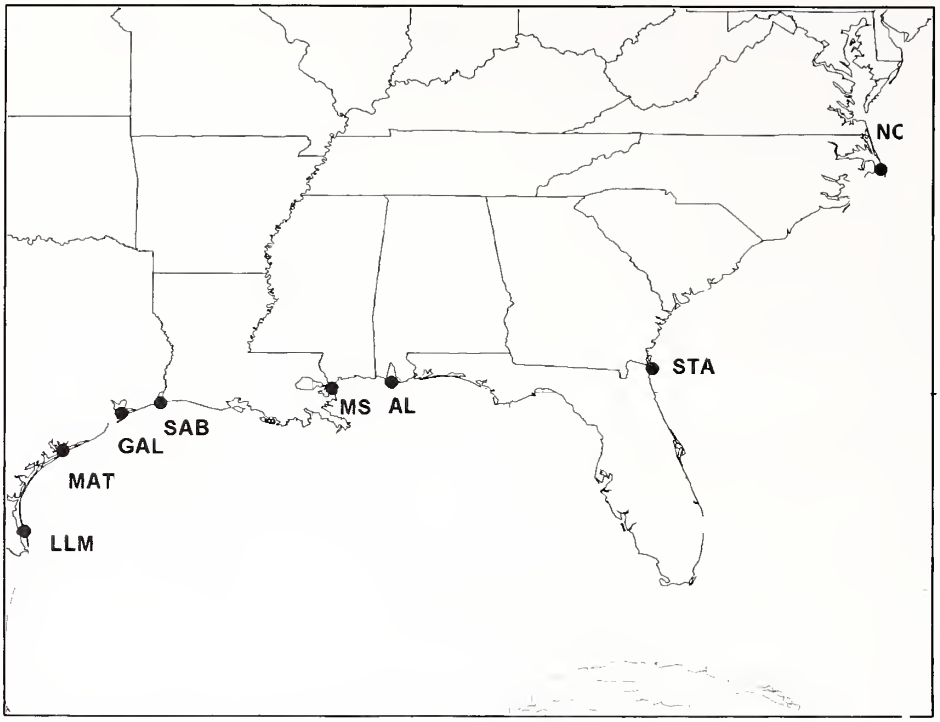

Figure 1

Collection sites for southern flounder in North American waters. LLM = Lower Laguna Madre; MAT = Matagorda Bay; GAL = Galveston Bay; SAB = Sabine Lake; MS = Mississippi; AL = Alabama; STA = St. Augustine, Florida; NC = North Carolina.

as current patterns (King et al., 1994). Support for competing models comes from examination of specific patterns observed in structured populations. Such patterns, if observed, may have important management implications.

Materials and methods

Southern flounder were collected during the summers of 1996 and 1997 by rod and reel, flounder gigs, or Texas Parks and Wildlife (TPW) gill nets in four Texas bays (Sabine Lake, Galveston Bay, Matagorda Bay, and lower Laguna Madre), by pound nets in Core Sound, North Carolina, from gill nets in estuarine waters near Biloxi, Mississippi, and from commercial fish houses in Dauphin Island, Alabama, and St. Augustine, Florida (Fig. 1). Southern flounder from commercial fish houses were reported by the house operator to be caught locally. A majority of individuals collected were adult and were not reliably assignable to year classes. Samples were screened by using isoelectric focusing (IEF) of sarcoplasmic proteins (methods of Ward et al., 1995) to insure that individuals belonging to other *Paralichthys* species were not included in our analyses (necessary because of accidental inclusion of congenerics both in samples obtained from commercial sources and in samples of juvenile flounder obtained during routine resource sampling in Texas).

Skeletal muscle, liver, heart, and kidney tissues were excised from fresh or frozen fish. Sample preparation and electrophoretic techniques and conditions followed those of King and Pate (1992). Gel and electrode buffers used were tris-borate-EDTA, pH 8.0 (Selander et al., 1971), tris-citrate, pH 8.0. (Selander et al., 1971), lithium hydroxide, pH 8.0 (Selander et al., 1971), borate buffer, pH 9.0 (modified from Sackler, 1966), and Poulik's discontinuous system, pH 8.7 (Selander et al., 1971). Histochemical methods were primarily taken from Manchenko (1994). Genetic nomenclature followed the recommendations of Shaklee et al. (1990).

BIOSYS-1 (Swofford and Selander, 1981) was used to generate an allele frequency table, to estimate the proportion of loci heterozygous (*H*) in the average individual, the proportion of polymorphic loci in individuals from each bay population, and genetic divergence using the *F*-statistics of Wright (1978). Exact tests calculated by GENEPOP (v. 3.1; Raymond and Rousset, 1995) were used to test for conformance of genotypic frequencies at each locus within a sample to Hardy-Weinberg expectations, genotypic linkage disequilibrium, and allelic and genotypic heterogeneity. Pairwise differences between samples were tested by using the genic differentiation randomization test in GENEPOP. Results were combined over loci with Fisher's method (Sokal and Rohlf, 1994). Differences between each pair of populations were summarized by using the chord distance of Cavalli-Sforza and Edwards (1967). An unrooted phylogenetic tree was fitted to the chord distance matrix by using the neighbor-joining (NJ) algorithm. TreeView (Page, 1996) was used to visualize the tree. The strength of support for each node in the tree was tested by bootstrapping over loci with NJBPOP (Cornuet et al., 1999). To further quantify spatial heterogeneity, the fixation index (F_{ST}) was calculated for each locus to provide measures of interpopulation differentiation and estimates of reduction in heterozygosity of a subpopulation due to population subdivision. A χ^2 test was used to test the null hypothesis, $F_{ST}=0$ (Workman and Niswander, 1970). All χ^2 probability values from tests for conformance to Hardy-Weinberg expectations, heterogeneity, linkage disequilibrium, and F_{ST} were adjusted for multiple simultaneous tablewide tests by using sequential Bonferroni adjustments to minimize type-I statistical inference errors (Rice, 1989).

Partitioning of variance components among geographic regions and within samples was accomplished by using a hierarchical analysis of molecular variance (AMOVA, Cockerham, 1969, 1973) with the package ARLEQUIN (version 2, Schneider et al., 1999). Sample sites were nested into regional groupings for separate analyses (Atlantic versus Gulf of Mexico, and Atlantic combined with eastern Gulf sites versus western Gulf). A phenogram was generated from the chord distance matrix with the neighbor-joining (N-J) algorithm. The N-J phenogram, with bootstrap estimates (as percentage of 10,000 replications) obtained by resampling loci within samples, was generated with NJBPOP (Cornuet, et al., 1999). The significance of the relationship between genetic (i.e. chord) and geographic (bay to bay shoreline distance) distance matrices was determined by sampling the randomization distribution generated from 1000 replications with the MXCOMP (matrix comparison) routine in NTSYS-PC 2.0 by Rohlf (1997) to allow a Mantel test (Mantel, 1967). An assignment test (WHICHRUN 4.1, Banks and Eichert, 2000) tested the ability to discriminate population of origin based on an individual's multilocus genotypic profile.

Clinal trends in heterozygosity and allele frequency were examined by using nonparametric correlation analyses (SAS Institute, 1989). Significance of Spearman's correlation coefficients was determined as the probability a correlation differed from zero. Probabilities less than 0.05 were considered statistically significant (Snedecor and Cochran, 1980).

Results

Misidentifications detected by IEF resulted in a reduced sample size in some samples especially from Alabama and Florida. Examination of 46 enzymes and structural protein systems in southern flounder produced scorable phenotypes for 68 putative gene loci. Two dimeric esterase loci (*ESTD-1** and *ESTD-2**, 3.1.1.. [IUBMBNC, 1992]) a tripeptide aminopeptidase locus (*PEPB-2**, 3.4.13..), glycerol-3-phosphate dehydrogenase (*G3PDH**, 1.1.1.8), two glucose-6-phosphate isomerase loci (*GPI-A** and *GPI-B**, 5.3.1.9), phosphoglucomutase (*PGM**, 5.4.2.2) and two glucose-6-phosphate dehydrogenase loci (*G6PDH-1** and *G6PDH-2**, 1.1.1.49) were resolved, scored as variable, and included in analyses. The remaining 59 loci were monomorphic or could not be scored consistently and were omitted. All polymorphic loci had the same common allele across all localities (Table 1). *ESTD-1*, ESTD-2*, GPI-B**, and *G6PD-1** each expressed an allele unique to a single locality. The percentage of polymorphic loci ($P_{0.99}$) averaged 7.5% and ranged from 4.41% to 10.29% (Table 1). Mean individual heterozygosity ranged from 0.03 (SE=0.02) in North Carolina and Florida to 0.12 (SE=0.12) in Matagorda Bay. Statistically significant clinal relationships were found in the Gulf of Mexico between the frequency of the common allele of the *G6PDH-2** locus and degrees west longitude ($r_s=-0.829$, $P<0.05$) and degrees north latitude ($r_s=0.829$, $P<0.05$). Further differentiation of populations in the western Gulf of Mexico was observed at the *G3PDH** locus. The *G3PDH*1* allele occurred at a frequency of 12.0% in the Laguna Madre, declined to a frequency of 1.4% in Galveston Bay, and was absent in all collections east of Galveston Bay. Four loci had alleles confined to a single locality in this same region of Gulf of Mexico (Table 1), including *G6PDH-1*2* which was limited to the Laguna Madre. Samples were in Hardy-Weinberg equilibrium at the nine allozyme loci surveyed for each of the 72 possible comparisons of variable loci for each locality, except *ESTD-2** in Galveston Bay. No statistically significant genotypic linkage disequilibrium was observed between any loci for any population.

Tests for homogeneity of allelic frequencies at variable loci across all localities were statistically significant at six of the nine loci: *ESTD-2** ($P<0.01$), *G3PDH** ($P<0.01$), *GPI-A** ($P=0.01$), *GPI-B** ($P<0.01$), *PGM** ($P<0.01$), and *G6PDH*-2* ($P<0.01$). Tests for homogeneity of genotypic frequencies across all localities were significant at four loci: *G3PDH** ($P<0.01$), *GPI-B** ($P<0.01$), *PGM** ($P<0.01$), and *G6PDH-2** ($P<0.01$). F_{ST} averaged 0.088 and varied from 0.181 for *G6PDH-2** to 0.001 for *G6PDH-1**. All estimates of F_{ST} were found to be statistically different from 0, except for loci *G6PDH-1**, *PEPB-2**, and *GPI-A**.

Regional differentiation among southern flounder was demonstrated (Fig. 2) by using chord distance and N-J clustering. The greatest discontinuity was between all samples from Galveston Bay eastward and a cluster composed of Matagorda Bay and the Laguna Madre. No apparent differentiation exists between Atlantic Coast populations and populations collected from the eastern Gulf of Mexico. This pattern is supported by the outcome of the assignment test (Table 2). Percentage correct assignment ranged from 0% for Florida to 78% for Matagorda Bay. However, when assignments between groups identified in the cluster analysis were examined, the overall correct percentage increased to 81%. The assignment test supports the interpretation of the cluster analysis, suggesting within-region differentiation was minimal and not geographically consistent, but between-region differences were considerable. A different pattern was discerned by using AMOVA (Table 3). The majority of the variance (>99%) was distributed within samples and the among-sites-within-coasts component was nonsignificant. Comparison of Atlantic versus Gulf of Mexico sample sites was statistically significant ($P=0.04$) and comparison of western Gulf of Mexico sample sites with those from the east-

Table 1

Allele frequencies, sample sizes (*n*), percentage of polymorphic loci per population, and mean heterozygosity per locus. LM = Laguna Madre, Texas; MAT = Matagorda Bay, Texas; GAL = Galveston Bay, Texas; SAB = Sabine Lake, Texas; MIS = off Biloxi, Mississippi; ALA = Mobile Bay, Alabama; STA = off St. Augustine, Florida; NC = Core Sound, North Carolina.

Locus	Allele	LM	MAT	GAL	SAB	MIS	ALA	STA	NC
ESTD-1*[1,3]	*1	0.972	0.918	0.889	0.917	0.885	1.000	1.000	0.959
	*2	0.028	0.082	0.111	0.083	0.115	0.000	0.000	0.027
	*3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
	n	54	61	36	24	13	21	20	37
ESTD-2*[1,3]	*1	0.009	0.000	0.014	0.000	0.000	0.000	0.000	0.054
	*2	0.981	1.000	0.917	1.000	1.000	1.000	1.000	0.946
	*3	0.009	0.000	0.069	0.000	0.000	0.000	0.000	0.000
	n	54	61	36	24	13	21	20	37
PEPB-2*[1,3]	*1	0.074	0.016	0.014	0.000	0.038	0.024	0.025	0.000
	*2	0.926	0.984	0.986	0.958	0.962	0.976	0.975	1.000
	*3	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000
	n	54	61	36	24	13	21	20	37
G3PDH*[2,3]	*1	0.120	0.098	0.014	0.000	0.000	0.000	0.000	0.000
	*2	0.778	0.779	0.972	0.848	0.808	0.833	0.875	0.946
	*3	0.102	0.123	0.014	0.152	0.192	0.167	0.125	0.054
	n	54	61	36	23	13	21	20	37
GPI-A*[1,3]	*1	1.000	0.992	1.000	1.000	0.962	0.929	0.975	1.000
	*2	0.000	0.008	0.000	0.000	0.038	0.071	0.025	0.000
	n	54	61	36	24	13	21	20	36
GPI-B*[1,4]	*1	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000
	*2	1.000	1.000	0.986	0.875	1.000	1.000	1.000	1.000
	*3	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	n	54	61	36	24	13	21	20	37
PGM*[1,4]	*1	0.009	0.008	0.086	0.000	0.000	0.000	0.000	0.000
	*2	0.991	0.992	0.829	1.000	1.000	1.000	1.000	1.000
	*3	0.000	0.000	0.086	0.000	0.000	0.000	0.000	0.000
	n	54	61	35	24	13	21	20	37
G6PDH-1*[1,3]	*1	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*2	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	n	54	61	36	24	13	21	20	37
G6PDH-2*[1,3]	*1	0.000	0.000	0.114	0.000	0.000	0.095	0.025	0.000
	*2	0.639	0.648	0.871	0.896	0.962	0.881	0.975	1.000
	*3	0.361	0.352	0.014	0.104	0.038	0.024	0.000	0.000
	n	36	61	35	24	13	21	20	37
% polymorphic loci		10.29	8.82	10.29	7.35	7.35	5.88	5.88	4.41
Heterozygosity		0.10	0.12	0.09	0.08	0.08	0.09	0.03	0.03
Direct count (SE)		(0.05)	(0.06)	(0.04)	(0.03)	(0.03)	(0.04)	(0.02)	(0.02)

[1] Buffer = TVB (Tris-borate-EDTA, pH 8.0).
[2] Buffer = borate 9.
[3] Tissue = muscle.
[4] Tissue = liver.

ern Gulf and the Atlantic coast approached significance (P=0.07). The results of chi-square tests for population differentiation (Table 4) allowed a pairwise examination of differences among the eight sampling sites. Eastern sample sites were not significantly different (P>0.01) from their nearest geographic neighbors. Western sites, from Sabine Lake to the lower Laguna Madre, were statistically different from at least one neighbor, suggesting more pronounced genetic differentiation in the western Gulf of Mexico. Among Gulf of Mexico samples, no sta-

Table 2

Assignment test outcomes. Values indicate frequency of assignment of individuals from a collection locality (rows) to the locality to which it is most similar (columns). Numbers on the diagonal indicate correct assignments. The "East" category combines collections from North Carolina to Sabine Lake, Texas, and the "West" category combines Texas sites from Galveston Bay to Laguna Madre. NC = Core Sound, North Carolina; STA = off St. Augustine, Florida; ALA = Mobile Bay, Alabama; MIS = off Biloxi, Mississippi; SAB = Sabine Lake, Texas; GAL = Galveston Bay, Texas; MAT = Matagorda Bay, Texas; LM = Laguna Madre, Texas.

	Assigned to							
From	NC	STA	ALA	MIS	SAB	GAL	MAT	LM
NC	29	0	4	0	2	0	0	0
STA	13	0	5	1	0	1	0	0
ALA	8	0	6	3	0	0	0	0
MIS	4	1	0	4	3	1	0	0
SAB	15	1	4	2	10	5	0	0
GAL	9	0	2	1	2	22	1	0
MAT	8	0	6	6	1	0	14	25
LM	7	0	4	0	0	1	3	19
East			158					1
West			33					61

tistically significant correlations were observed between chord distances and geographic distances (r=0.416, t=1.549, P=0.06).

Discussion

Genetic differentiation was not extensive over most of the range of *P. lethostigma* examined in this study. Samples collected from Core Sound in North Carolina to Sabine Lake on the upper Texas coast were genetically similar. However, a discontinuity in allele frequencies was identified on the Texas coast between Galveston and Matagorda Bays. In addition, statistically significant clines in allele frequencies at the *G6PDH-2** locus and in average individual heterozygosity were observed across the Gulf of Mexico. These observations do not suggest the occurrence of independent stocks of southern flounder in the Gulf of Mexico but do support the hypothesis that genetic structuring is present. Southern flounder samples collected off St. Augustine, FL, and off North Carolina cluster with samples in the Gulf of Mexico from Galveston Bay, Texas, eastward, despite a modern-era distributional gap that encompasses the southern reaches of Florida from the Loxahatchee River on the Atlantic Coast to the Caloosatchee Estuary on the Gulf Coast (NOAA[5]). This apparent gap may not represent an effective barrier to gene flow or may be of such recent origin that differentiation has been minimal. It is also possible that differences existed that were undetected by techniques used in our study.

Significant correlation between genetic and geographic distance was not found, lending no support to application of the isolation by distance model (Wright, 1943) to pop-

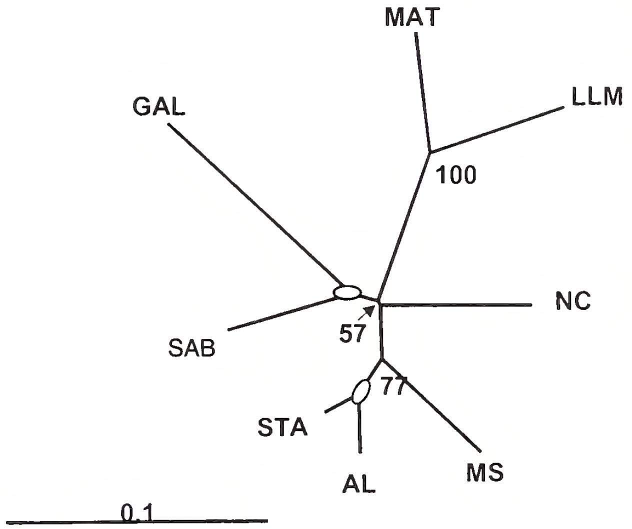

Figure 2

Unrooted neighbor-joining tree depicting the underlying structure found in the pairwise Cavalli-Sforza and Edwards chord distance matrix for eight collections of southern flounder. Numbers indicate bootstrap support in 1000 replications and white ellipses indicate nodes with bootstrap support below 50%. LLM = Lower Laguna Madre; MAT = Matagorda Bay; GAL = Galveston Bay; SAB = Sabine Lake; MS = Mississippi; AL = Alabama; STA = St. Augustine, Florida; NC = North Carolina.

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[5] NOAA. 1985. Gulf of Mexico coastal and ocean zones strategic assessment:data atlas, 188 p. Strat. Assess. Branch, Ocean Assess. Div., Off. Oceanography Mar. Assess., Nat. Ocean Serv. and the Southeast Fish. Center, NMFS, NOAA, U.S. Dep. Commer., 75 Virginia Beach Dr, Miami, FL 33149.

ulation structuring in southern flounder. The estimated F_{ST} value of 0.088 for southern flounder was greater than that found for *Sciaenops ocellatus* (F_{ST}=0.022, Gold et al., 1994), *Cynoscion nebulosus* (F_{ST}=0.009, King and Pate, 1992), or *Pogonius chromis* (F_{ST}=0.013, Karel, unpubl. data) in this region. Physical or biotic factors may have caused greater isolation for southern flounder than for other nearshore species. Cluster analysis suggested that the region of greatest differentiation occurs along the middle Texas coast. Similar genetic structure in *Crassostrea virginica* may be explained by seasonal current patterns in Corpus Christi Bay, Texas (King et al., 1994). A comparable mechanism may operate in southern flounder; offshore currents may have resulted in reduced dispersion of eggs and larvae between the upper and middle Texas coasts. Currents off the Texas coast are seasonally variable and complex (Cochrane and Kelly, 1986) and may aid in reducing egg and larval dispersion between regions of the western Gulf of Mexico.

Table 3

Hierarchical analyses of molecular variation (AMOVA) among mtDNA composite haplotypes of southern flounder (*Paralichthys lethostigma*) from the U.S. Atlantic coast and Gulf of Mexico.

Source of variation	Variance	% variation	P^1
All sites			
Among coasts	0.00026	0.06	0.04
Among sites within coasts	–0.00020	–0.04	0.92
Within sites	0.44985	99.99	0.74
Eastern versus western cluster			
Among clusters	0.00042	0.09	0.07
Among sites within regions	–0.00034	–0.08	0.10
Within sites	0.44985	99.98	0.76

[1] Probability of finding a more extreme variance component by chance alone (1000 permutations).

Many marine species spawn in the open ocean, have eggs and larvae with an extensive planktonic stage, or are highly mobile as adults. It is not surprising that such organisms are often panmictic, or exhibit subdivision on only broad levels (e.g. *Sciaenops ocellatus*; Gold et al., 1994). When genetic differentiation is found in marine organisms (e.g. *Cynoscion nebulosus*; King and Pate, 1992), extensive regions of clinal change in allele frequency may be seen and may be an adaptive feature (King and Zimmerman, 1993). A critical question for fishery management is how much differentiation is necessary to indicate biologically significant population structuring. Gold et al. (1994), for instance, found that red drum were subdivided (albeit weakly) between Atlantic and Gulf of Mexico subpopulations despite relatively high levels of gene flow between the populations and failure of cluster analyses to consistently segregate localities into proper geographic regions. However, significant differences in allele frequencies for two loci were found and the researchers were able to demonstrate, through hierarchical gene-diversity analysis, that 20% of the variation in gene diversity was related to within-region differences.

Table 4

Tests (χ^2) of pairwise population differentiation for southern flounder (*Paralichthys lethostigma*) samples obtained from sites on the U.S. Gulf and Atlantic coasts based on variability at eight allozyme loci. The χ^2 value tests the hypothesis of no difference in allelic distribution across collection localities. The associated probability (P) is given in parentheses. Inf. = infinite.

	North Carolina	Florida	Alabama	Mississippi	Sabine	Galveston	Matagorda
Florida	12.875 (0.38)						
Alabama	22.791 (<0.01)	4.478 (0.81)					
Mississippi	28.820 (<0.01)	13.350 (0.21)	7.723 (0.66)				
Sabine	26.087 (0.01)	17.208 (0.14)	18.942 (0.09)	21.959 (0.04)			
Galveston	59.146 (<0.01)	40.903 (<0.01)	42.314 (<0.01)	37.970 (<0.01)	44.407 (<0.01)		
Matagorda	Inf. (<0.01)	Inf. (<0.01)	Inf. (<0.01)	Inf. (<0.01)	Inf.Inf. (<0.01)	(<0.01)	
Laguna Madre	Inf. (<0.01)	Inf. (<0.01)	Inf. (<0.01)	42.178 (<0.01)	Inf. (<0.01)	Inf. (<0.01)	26.956 (0.07)

In our study, allele frequency discontinuities and clinal variation in genetic characters were identified among southern flounder inhabiting the western portion of the species' range. Short-term goals (e.g. supplementing exploited populations) that fail to account for this structuring could result in management programs that undermine the long-term resource management objective: maintaining the evolutionary potential of this species. The results of this survey suggest that southern flounder populations should be considered potentially distinct pending further resolution of population differentiation and clinal variation. Stocking efforts, especially those involving interbay transfers, should be undertaken only after careful consideration of all pertinent information, and be contingent upon careful studies of genetic variation at the appropriate local level. Observed discontinuities and clinal variations in allele frequencies may indicate adaptation to localized conditions and should be incorporated into comprehensive management strategies developed for southern flounder.

Acknowledgments

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Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks

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The common octopus (*Octopus vulgaris* Cuvier, 1797) is an Atlantic and Mediterranean species (Guerra, 1992; Mangold, 1998). It is one of the most important target species of the Northwest African fisheries (Hernández and Bas, 1993; Foucher et al., 1998). The common octopus catch reported for this area in 1994 was 137,844 t, representing 47.17% of the total world octopus catch. In 1996 it was 156,300 t, representing 50.03% of the total world octopus catch (FAO, 1998).

Octopus age and growth have been determined by laboratory rearing studies (Itami et al., 1963; Nixon, 1969; Mangold and Boletzky, 1973; Smale and Buchan, 1981; Villanueva, 1995) and by field studies (Guerra, 1979; Hatanaka 1979; Pereiro and Bravo de Laguna, 1979). Growth rates can be calculated for animals maintained in the laboratory, but comparison with growth under natural conditions is questionable (Mangold, 1983). In field studies, growth and age can be correlated when there is clear evidence that a single year class from a stable population is under consideration, but where the spawning season is very long as in the common octopus (Mangold, 1983; Guerra, 1992), identifying year classes is difficult (Guerra, 1979, Hatanaka, 1979).

Cephalopod age has been determined by several methods: Guerra (1979), Pereiro and Bravo de Laguna (1979), and others have reported growth and age correlations by following a single year class from a stable population. Concentric rings on statoliths (Young, 1960), the internal shell, and eye lenses (Gonçalves, 1993) of *Octopus vulgaris* have been reported. Raya and Hernández-González (1998) observed marks on the internal rostral area of beaks from common octopus, possibly related to daily growth.

None of these methods has been validated for known-age *Octopus vulgaris*. Furthermore, all require fairly complex methods for preparing structures prior to observation under the microscope (polishing, embedding in resin, and sectioning with a diamond, etc.) which hinders their application to field studies. This paper provides an easy new method of determining *Octopus vulgaris* age based on the upper beak microstructure, validated for the paralarval period.

Material and methods

The study was carried out on 275 common octopus (164 males and 111 females) collected between January 1998 and May 1999, from catches of the small-scale fishery off the island of Gran Canaria (The Canary Islands, central-east Atlantic).

An additional sample of 27 *Octopus vulgaris* paralarvae was obtained from spawning females that deposited and incubated egg bunches in plastic burrows inside a 12,000-L tank. The embryonic development took between 25–30 days at a temperature range of 19–22°C. Once hatched, the paralarvae were transferred to transparent 12-L containers with open seawater flow, in July 1997 and June and July 1999, and reared in the laboratory at 19–22°C water temperature and natural photoperiod. The dates of hatching and death of each paralarva were recorded. The bottom was siphoned daily to remove dead individuals. During rearing, paralarvae were fed with recently hatched crab zoeae (see Hernández-García et al., 2000).

Ventral mantle length (VML) was measured in both benthic octopus and paralarvae to the nearest 0.1 mm. We used VML as the body measurement because we consider it to be more accurate than dorsal mantle length. Total body weight (TW) of benthic octopus was recorded to the nearest 0.01 g (to the nearest 0.0001 g in paralarvae). With the exception of the paralarvae, all the specimens were sexed.

The beak of each animal, including paralarvae, was removed and stored in 70% ethyl alcohol. Lower and upper beaks were sagittally sectioned with scissors to obtain two symmetrical half beaks (Fig. 1). The half beaks were cleaned with water and the mucus covering the inner part of the lateral walls was removed by rubbing it softly with the fingers (obviously, this operation was not necessary in the case of the paralarvae beaks).

By using a stereoscopic microscope, the concentric rings in the lateral wall of each beak were counted from the rostral tip area to the opposite end of the lateral wall. Because of the lack of pigmentation in paralarvae beaks, the concentric rings in their lateral wall were more easily counted with a microscope. Rings of each beak were

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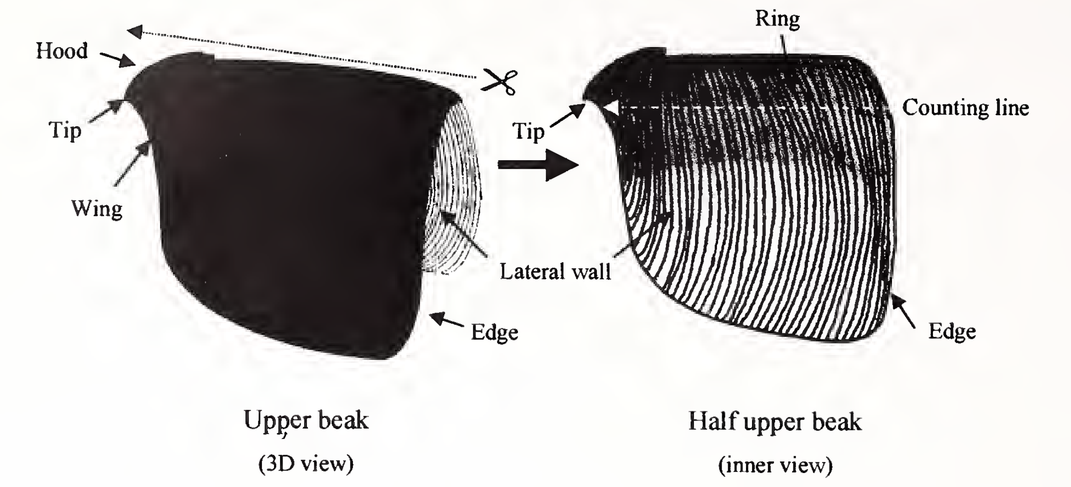

Figure 1

Diagram of upper beak of *Octopus vulgaris* and lateral wall showing growth bands, rostral tip area, the distal posterior beak end, and counting line.

counted at least three times by the same person, and those with less than two identical counts were rejected from analysis.

The number of rings in beaks of paralarvae was compared with the number of days each one lived.

Results

Octopus obtained from the small-scale fishery ranged in size from 4.8 to 165 mm VML, and weighed between 0.38 and 3926 g. Females ranged from 60 to 165 mm VML and weighed from 215 to 3926 g. Males ranged from 58 to 160 mm VML and from 200 to 3167 g in weight. We were unable to sex two individuals (4.8 mm VML, 0.38 g and 8.1 mm VML, 0.60 g). Paralarvae ranged from 1.0 to 2.7 mm VML and from 0.001 to 0.005 g in weight.

The internal lateral walls of upper beaks from 302 individuals (27 paralarvae and 275 individuals in benthic stages of *Octopus vulgaris*) revealed a pattern of concentric bands deposited from the rostral tip of the beak to the opposite margin of the lateral wall, parallel to the beak edges. Both halves of the upper beaks showed similar spatial and density patterns of microstructures. Lower beaks showed no regularity in the pattern of bands along the lateral walls and were discarded. On the upper beaks the distance from the rostral tip to the distal end of the lateral wall showed a positive correlation with the VML (Pearson's correlation: r=0.825; P<0.001) (Fig. 2).

Ring counts were more difficult near the rostral tip where rings were frequently discontinuous. Counts were easier to make near the edges of the lateral wall which was less highly pigmented.

Paralarvae survived in tanks from 3 to 26 days. For 48.1% of paralarvae, the concentric ring count in the lateral wall of the upper beak equaled the number of days that they lived. Otherwise, in 22.2% and 29.6% of paralarvae the number of rings counted were one more or one less, respectively, than the number of days of age. These data (Fig. 3) indicate that daily deposition of a growth increment in the lateral wall of the upper beak begins on day one after hatching. The weight-age and VML-age relationships of paralarvae (Fig. 4, A and B) were similar to those found by Villanueva (1995), with differences attributable to rearing conditions. Given the correlation between increment counts and age of paralarvae, we applied the upper beak ring count method for age determination of 272 common octopus, ranging from 4.8 to 165 mm VML. Results should be taken with caution for the benthic stages of octopus pending the validation of growth of adults and the frequency of rings deposition.

Increments counted on beaks from octopus collected in the wild ranged from 53 to 398 corresponding to individuals of 0.38 and 3926 g body weight, respectively (4.8 and 165 mm VML). Males and females had no difference in the number of rings counted in the lateral walls of upper beaks (ANOVA, F=0.0006, P=0.98). The age of males ranged between 3.2 and 12.3 months (95–369 rings), and females ranged between 3.1 and 13.3 months (93–398 rings), see Figure 5 (A and B) for weight-age and MVL-age relationships for benthic octopus.

Discussion

In *Octopus vulgaris* and other shallow-water cephalopods, regular patterns of activity and evidence of endogenous rhythms induced by the light–dark cycles have been reported in both field and laboratory animals (Cobb et al., 1995). These endogenous rhythms may be reflected in a

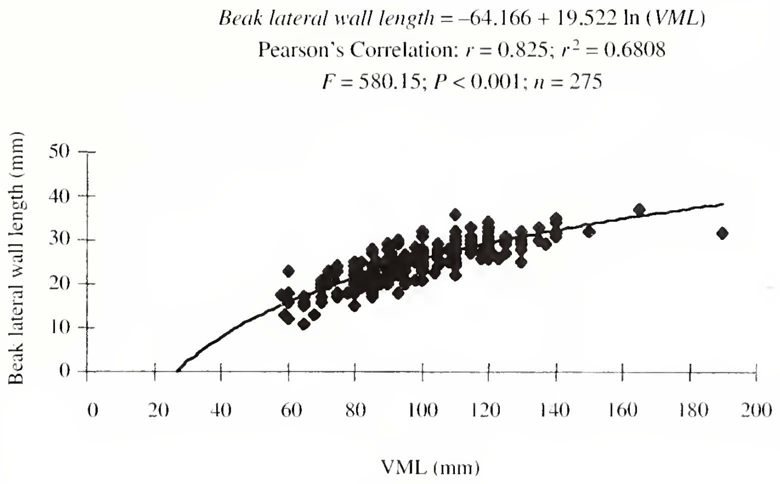

Figure 2

Relationship of beak lateral wall length to ventral mantle length (VML) of benthic *Octopus vulgaris*.

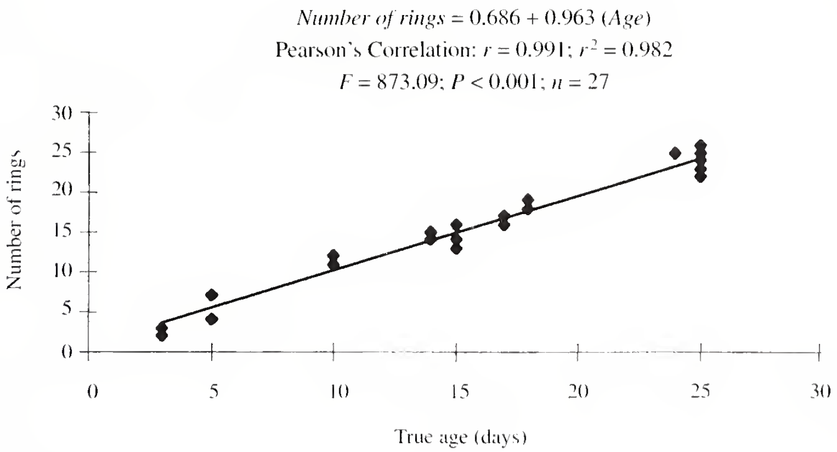

Figure 3

Lateral wall ring counts versus real age in 27 paralarvae from 3 to 26 days old.

chitinous structure such as the beaks (Raya and Hernández-González, 1998) or in calcium deposits in statoliths. Statoliths are the hard structures most commonly used for cephalopod age estimation (Lipinski, 1986, 1993; Arkhipkin, 1993), although the presence of concentric rings in the internal shell, beaks, and eye lenses have also been used (Clarke, 1965; Gonçalves, 1993; Raya and Hernández-González, 1998). When beaks are used, erosion of the rostral area during the life of the animal may bias age determination toward underestimation (some of the first rings may be eroded and therefore not counted). We found evidence of incomplete increments on the edge of the lateral wall, near the rostral tip area; therefore, ages we provide for benthic adults are to be considered minimum estimates.

If rings on the lateral walls of the upper beaks are laid down daily and can be accurately counted even in the oldest specimens (as indicated by the pattern in paralarvae), then our results are consistent with a lifespan of 12–13 months in the Canary Island waters. Raya and Hernández-González (1998) gave a lifespan of 10–12 months for octopus caught off the coast of northwest Africa (21–26°N)

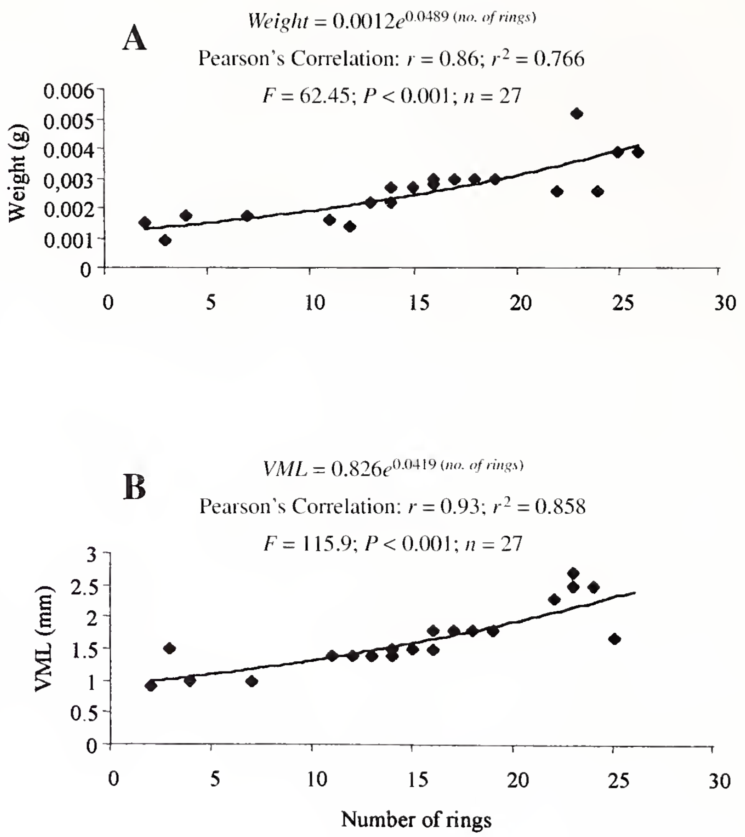

Figure 4

Plot of the number of rings counted on the lateral walls of the upper beak against (**A**) body weight and (**B**) ventral mantle length (VML) of paralarvae of *Octopus vulgaris*.

although they reported some heavier but younger specimens than we found. This difference could be due to discrepancies in the aging methods or, as in the case of Mangold (1983), areas off the coast may have different growth patterns and lifespans. Thus, Smale and Buchan (1981) proposed a lifespan of 9–12 months in females and 12–15 in males *Octopus vulgaris* from the South African coast.

Several authors have noted that size (and probably weight) may not reliably indicate age in field-caught cephalopods (Mangold and Boletzky, 1973; Hixon, 1980) because it may vary greatly depending upon factors such as food and temperature (Van Heukelem, 1979; Mangold, 1983). Cephalopods reveal great morphological variability with latitude attributed to environmental influences on development (Hernández-García and Castro, 1998), and probably on lifespan. The length and weight ranges of octopus caught off the Canary Islands are within the ranges reported for this species off East Africa, are the limits of range (upper and lower) off South Africa (Smale and Buchan, 1981) and in the western Mediterranean Sea (Mangold, 1983), although the range recorded in our study (Canary Islands) is closer to that reported for the Mediterranean Sea.

The smallest octopi that we examined from fishery catches were 4.8 and 8.1 mm VML (0.38 and 0.60 g TW, respectively), well outside the minimum commercial length (90–100 mm VML). Their estimated ages were 51 and 91 days old. In the English Channel, the planktonic phase for common octopus has been estimated at 3 months (Rees, 1950; Rees and Lumby, 1954); and average weight of 0.2 g at settling may be normal regardless of temperature (Mangold, 1983). *Octopus* typically spend the first 5–12 weeks of life as an active predator on plankton (Mangold, 1983); they change gradually from a planktonic to benthic life style (Boletzky, 1977) in some way dependent upon temperature (Mangold 1983). We did not observe marked differences in ring pattern spacing indicative of the transition between planktonic and benthic life styles. However, the distance between rings does change during the benthic phase of life—a feature that seems related to water temperature—the rings being larger than average during winter and smaller during summer.

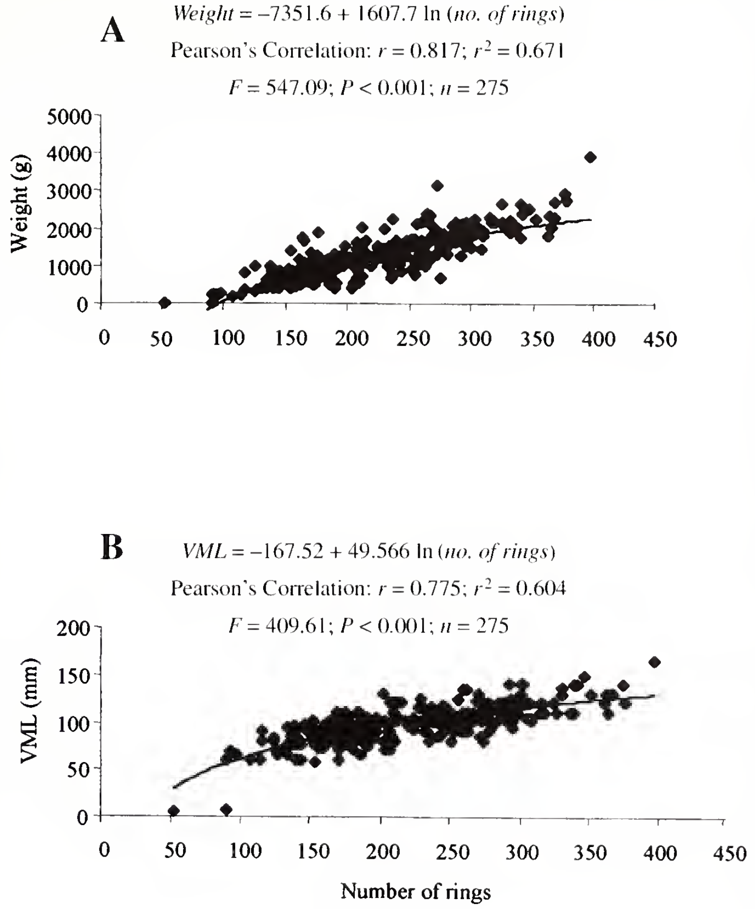

Figure 5

Plot of the number of rings counted on the lateral walls of the upper beak against (**A**) body weight and (**B**) ventral mantle length (VML) of benthic *Octopus vulgaris*.

Acknowledgments

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Reproduction of female spiny dogfish, *Squalus acanthias*, in the Oslofjord

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The spiny dogfish (*Squalus acanthias*) is a relatively small shark with a characteristic spine in front of each dorsal fin. Its dorsal side is grayish and has sporadic white spots. Although it may reach a length of 160 cm, most individuals in the North Sea are in the range of 80–100 cm (Ford, 1921). It is distributed worldwide, absent only from tropical and polar regions (Compagno, 1984).

The spiny dogfish has been harvested for more than 100 years mostly for its oil-rich liver (Ketchen, 1986). At first, the oil was used for lamp fuel and as a lubricant in machines. The oil was later (during W.W.II) used as a source of vitamin A. Today the dogfish is valued as food in many countries (Gordon, 1986).

The reproduction cycle of the spiny dogfish begins with mature females bearing several large (over 40-mm) yellow eggs. As the eggs pass through the shell gland they are fertilized and become enclosed in a protective capsule (candle). The candle passes down the reproductive tract and comes to rest in the uterus. The embryos live off the large yolk sac attached under the gill region (Gilbert, 1981). As the embryos grow, they slowly absorb the yolk sack. Embryos that have completely absorbed the yolk sac may still remain in the uterus for some time before being born (Ford, 1921). According to Jones and Geen (1977), the embryos also bear an internal yolk sac which nourishes them for up to 2 months after birth. The dogfish reproductive cycle takes almost 2 years, one of the longest gestation periods of any living vertebrate (up to 24 months) (Ketchen, 1972; Nammack et al., 1985).

This shark, like other sharks, is very susceptible to overfishing, not only because of its long gestation period, but also because of slow growth, late maturity, and because it bears a small number of offspring (up to 15) (Nammack et al., 1985; Fahy, 1989). Extensive fishing since the early 1960s has led to a marked decrease in the North Sea stock. Fishing has also affected the population in the Oslofjord where the annual catch declined from 704 tons in 1979 to less than 300 tons per year during the 1990s (Official Statistics of Norway, 1996). We investigated dogfish reproduction in the Oslofjord by comparing the reproduction parameters of dogfish caught in 1987 and 1997. The focus of our study was to evaluate the reproductive parameters of spiny dogfish in the Oslofjord and to look for possible changes in these parameters.

Materials and methods

Sampling

Dogfish were sampled monthly off the Hvaler Islands throughout 1987 and 1997 in gill nets and by longline at depths ranging from 50 to 460 m (Fig. 1). The gill nets were composed of monofilament line (0.60 mm) with a mesh size of 285 mm. This large mesh size accounts for catches consisting mainly of larger dogfish (over 70 cm). The longline was composed of a 5-mm line connected to a 7/0 dogfish hook. The nets were usually checked every 24 hours while the longline was taken up after a few hours. The samples consisted of 132 females in 1987 and 101 females in 1997. Total length and weight were measured to the nearest 0.5 cm and 5 g, respectively, as described by Saunders and McFarlane (1993).

The same fisherman, fishing grounds and fishing gear were used in both sampling years, so that sampling bias was avoided. The fishing gear accounted for catches of dogfish that were mainly over 70 cm in length. There was a relatively small amount of unmarketable-size fish caught and the discard rates were approximately the same in both years.

Age determination

The first and second dorsal spines were removed from 217 dogfish. The remaining 16 dogfish had spines that were either missing, or broken to such an extent that age determination was not possible for these individuals. The spines were air-dried at least 1 week before being cooked for approximately 3 minutes each in tap water. The flesh around the base of the spine was then removed with a scalpel and tweezers. The cleaned spines were subsequently dipped in ethyl alcohol and then dried with a soft cloth. Next, the spines were viewed under an Olympus SZH 10 zoom stereo microscope and aged according to the method described by Ketchen (1975). For large individuals with worn spines, age was modified by Ketchen's (1975) correction curve for age

$$Y = 0.5097X^{2.55},$$

where X = the diameter of the spine base in millimeters; and
Y = the additional age of the spine due to it being worn.

Ketchen's correction curve is based on fish caught in the Strait of Georgia, British Colombia. It is quite likely that there are differences in juvenile growth in the respective dogfish populations. The result may therefore be bi-

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Fish. Bull. 99:685–690 (2001)

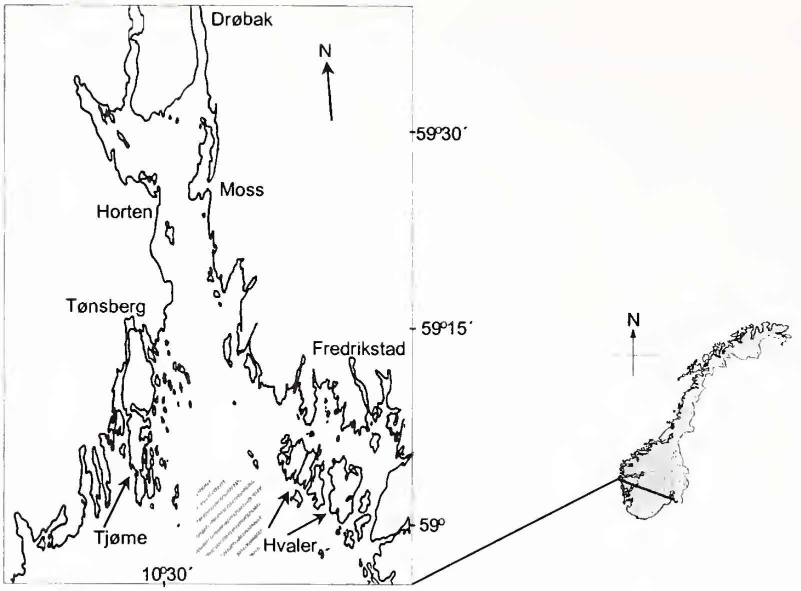

Figure 1

Map of the Oslofjord. The gray shaded area represents the capture location of dogfish in the outer Oslofjord.

ased and in future work a correction curve will have to be made for the stock in the Oslofjord.

Embryo development

Both ovaries of mature females were rinsed to expose eggs. The rinsing process consisted of removing and then carefully opening the ovaries with a scalpel. The eggs were counted and registered as belonging to the right or left ovary. In pregnant females the embryos were counted and sexed. The embryos were then measured to the nearest 0.1 cm on millimeter paper. Three stages of embryo development were identified according to Gauld[1]: stage 1 (candled embryos)—eggs, apparently fertilized, are present in a protective capsule in the uteri; stage 2 (free-living embryos)—the candle has ruptured and embryos bearing an external yolk sac are free in the uteri; and stage 3 (full term embryos)—fully developed embryos are present in the uteri. The yolk sac is fully absorbed and the umbilical slit is more or less closed.

Results

Biological parameters

The fish ranged in length from 54 to 110 cm in 1987 (mean 87 cm) and from 68 to 108 cm (mean 88 cm) in 1997. Weights ranged from 0.4 to 5.7 kg (mean 2.6 kg) in 1987 and from 1.1 to 5.5 kg (mean 2.9 kg) in 1997. Ages ranged from 9 to 35 years (mean 25 years) in 1987 and from 10 to 38 years (mean 23 years) in 1997.

On average, the fish in 1987 were 1.8 cm longer than fish in 1997 at the same age (P=0.02). However, there was a large variation in the length, and age could only account for 38% of this variability. Length accounted for 76% of the variability of the weight in 1987 and 93% in 1997. Fish between 80 and 110 cm were about 500 g heavier in 1997 (P<0.01). This difference was reflected in the condition factor, which was 3.8 in 1987 compared with 4.2 in 1997. This indicates that female dogfish caught in 1987 were longer and lighter than in 1997.

Growth

Growth appeared to be best represented by a linear growth equation for dogfish between the ages of 11 and 38 years, with an average annual increment of 0.7 cm per year (Fig. 2). There was no sign of reduction in growth with increasing age; that is, no asymptote was detected in these data.

Age and length at maturity

Maturity was defined as females bearing large ovarian eggs with a diameter of over 2 cm, candled young or free-living embryos. In both 1987 and 1997, most of the females matured between 12 and 26 years of age. PROBIT analysis indicated that dogfish reached 50% maturity at an age of 17.6 years in 1987 and 17.0 years in 1997 (Fig. 3). The

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[1] Gauld, J. 1979. Reproduction and fecundity of the Scottish-Norwegian stock of spurdogs, *Squalus acanthias* (L.). ICES, Council Meeting (CM) 1979/H:54, 13 p. Directory of fisheries, biblioteket, Pb. 185, 5001 Bergen.

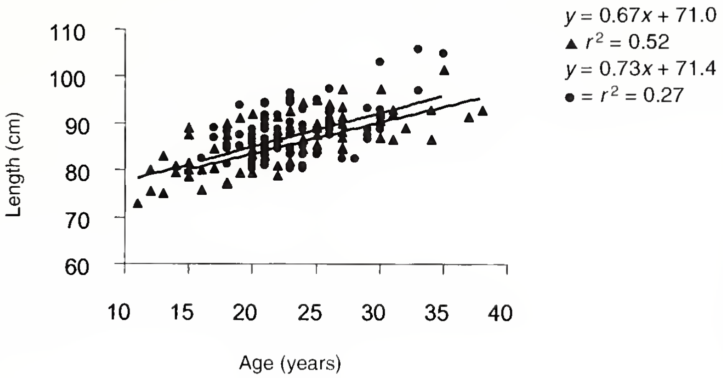

Figure 2

Growth of spiny dogfish in 1987 and 1997. ▲ = 1997 sample (n=79); ● = 1987 sample (n=92).

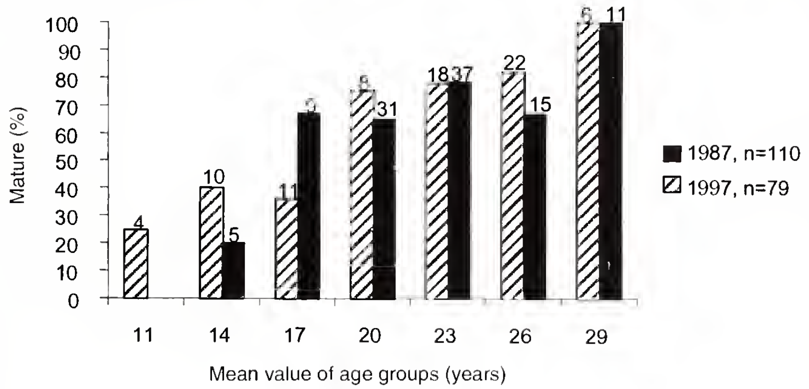

Figure 3

Percentage of mature fish in the different age groups 10–12, 13–15, 16–18,19–21, 22–24, 25–27 and 28–30 years in 1987 and 1997.

1997 sample also had a larger proportion of mature fish less than 15 years of age.

No individual was mature under 76 cm, but all individuals over 88 cm were mature, indicating that maturity occurs between 76 and 88 cm. The length at 50% maturity was 81 cm in both 1987 and 1997. There may be a difference in the growth rate between the two years; fish from 1997 reached maturity earlier than fish from 1987. However, because of the small sample size and difficulty with age determination, caution should be used to interpret this result.

Fecundity

In 1987 the number of ovarian eggs with a diameter over 2 cm (indicating mature fish) varied between 5 and 14 (mean=8.2, SD=2.2). In 1997 the range was 3–17 eggs (mean=8.9, SD=3). The relationship between egg number and adult length in the 1987 and 1997 samples was not significantly different. On average, the egg production increased by one egg per 4-cm adult length.

The number of free-living embryos in 1987 varied from 2 to 14 per female (mean=6.6, SD=2.7). In 1997, free-living embryos numbered between 3 and 15 (mean=7.5, SD=2.6). The increment of free-living embryos per adult length (Fig. 4) in the 1987 and 1997 sample was not significantly different. However, the level of the regression line was significantly higher in 1987 (P=0.04). On average, the females in 1987 carried 1.2 more free-living embryos. It should be emphasized that owing to the large variability, length could only explain 45% to 56% of the difference in the number of free-living embryos.

Reproduction cycle

Eggs on which the blastoderm was visible, were recognized as recently fertilized. This was part of the candle stage (from the newly fertilized eggs up to embryos 10 cm long) that lasted from October to November the following year. Fertilization occurred from the beginning of October to the beginning of February. Free-living embryos were observed from October to September of the following year, followed by parturation of fully mature embryos from September to December. The duration of the pregnancy for spiny dogfish in the Oslofjord ranged from 18 to 24 months. This period of time is calculated from the time of fertilization to the time of parturation. These estimates of the reproduction phases are summarized in Figure 5.

Embryonic growth

The embryos ranged from 4.4 to 24.9 cm total length. The growth of the combined 1986 and 1996 year classes of dogfish embryos was followed throughout their second year of development. Growth was approximately 1 cm per month for both year classes in the size range from 8 to 24 cm, which corresponds approximately to the embryos in the second year of development. However, growth was not uniform; it could be divided into two growth phases (Fig. 6). The first phase lasted from October to May with a slow growth of 0.6 cm/month. The second phase lasted from May until December and was characterized by a more rapid growth of 1.2 cm/month.

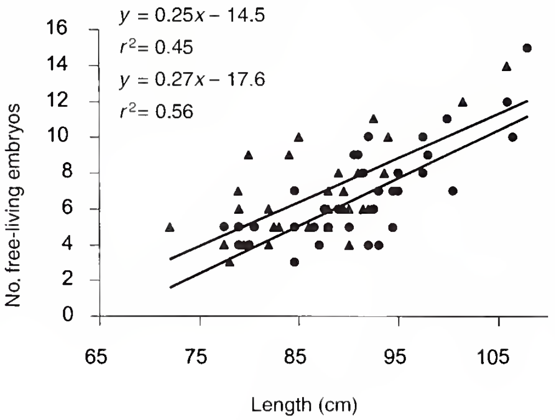

Figure 4

Number of free-living embryos in relationship to the length of the adult fish in 1987 and 1997. ▲ = 1987 sample; ● = 1997 sample.

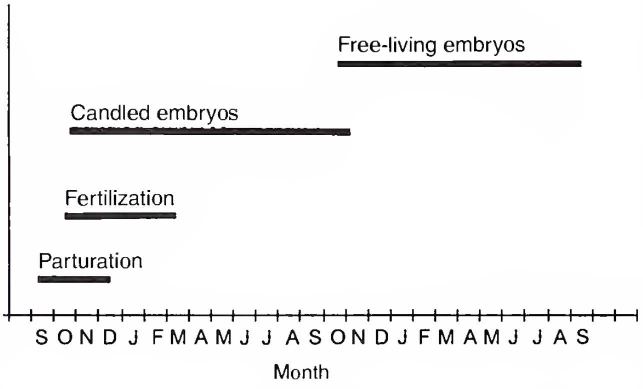

Figure 5

Schematic time representation of the reproductive cycle of the female spiny dogfish by month (n=194).

Discussion

Growth

The lack of an asymptote is probably due to an absence of older, larger fish. This is most likely due to over-harvesting of dogfish in the Oslofjord. According to local fishermen, heavy catches of large individuals (exceeding 110 centimeters) were taken in the late 70s and early 80s.[2]

Age and length at maturity

Age at 50% maturity was found to be 17.6 years in 1987 and 17.0 years in 1997. For spiny dogfish captured between the East Coast of Scotland and Norway, Aasen (1961) estimated age at 50% maturity to be in the range 12 to 14 years. In an area northwest of Scotland, Holden and Meadows (1962) reported age at 50% maturity of 9 years. However, Aasen (1961) and Holden and Meadows (1962) did not take into account worn spines, which combined with the difficulty of identifying all of the annual rings, means that their estimates are most likely biased downwards.

Length at 50% maturity in the Oslofjord should not deviate greatly from sizes observed elsewhere in the northeast Atlantic. Length at 50% maturity in the Oslofjord was found to be 81 cm in both 1987 and 1997. This is in general agreement with other observations in the northeast Atlantic where length at 50% maturity varied from 74 to 83 cm (Hickling, 1930; Holden and Meadows, 1964; Fahy, 1989; Gauld[1]).

Fecundity

There was no significant difference between the number of eggs observed in 1987 and 1997. However, the number of eggs in the ovaries was higher than the number of embryos in the uterus (average: 8.1 eggs and 6.6 free-living embryos in 1987 and 8.9 eggs and 7.5 free-living embryos in 1997). This is probably due to the absorption of eggs in the ovaries following fertilization that was observed by Hanchet (1988), Holden and Meadows (1964), and Nammack et al. (1985).

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[2] Johansen, P. A. 1997. Personal commun. Dogfish fisherman from the Oslofjord. Gudeberg Allè 1, 1600 Fredrikstad, Norway.

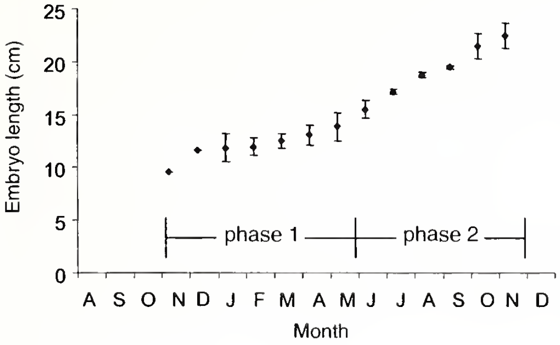

Figure 6

Length of the embryos during their second year of development for the combined 1987 and 1997 sample.

Fecundity was within the bounds found elsewhere in the northeast Atlantic (Aasen, 1961; Gauld[1]). Free-living embryos showed the same increase in number per length in 1987 and 1997, but the level was significantly lower in 1997 (on average one less embryo per unit of length). In addition the fish were shorter and heavier in 1997. However, these results must be taken with some caution because of the large variability in the data.

A small sample size and annual variability in both embryo production and food availability may have contributed to the lower fecundity in 1997. With regard to the sample sizes (31 and 38 fishes), it is emphasized that the covariation between the number of free-living embryos versus length was rather low. Although the differences between 1987 and 1997 were statistically significant, the overlap was so large that caution must be used in interpretation of the data.

Reproduction cycle and embryonic growth

In the Oslofjord, fertilization occurs from October to February and parturation from October to December. Research in other areas has shown that the pregnancy lasts 22–23 months (Ford, 1921; Gauld[1]). Thus, the duration of pregnancy in the Oslofjord (18 to 24 months) seems to have a greater variation than these observations. This variation may have been caused by the sample from the Oslofjord, which included dogfish that were fertilized early in the fertilization period, as well as dogfish that were fertilized towards the end of the fertilization period. This, in turn, indicates the possible minimal and maximal duration of pregnancy.

Behavioral factors may influence pregnancy duration, as well. Some dogfish may inhabit cooler water for longer periods of time than other dogfish, which may increase the duration of their pregnancy.

The differences in the embryo growth rate during their second year of development may be related to the water temperature. Hickling (1930) found that females migrate from deep to shallow water as pregnancy proceeds. This migration pattern exposes the embryos to different temperature levels that may influence embryonic growth, as found in Newfoundland by Templeman (1944). Embryos outside of Newfoundland had an average growth rate of 1.1 cm/month and a 24-month pregnancy period (Templeman, 1944). Outside of Woods Hole, Hisaw and Albert (1947) found an average growth rate of 1.3 cm/month and a pregnancy period of 20–22 months. Nammack et al. (1985) stated that the lower sea temperature outside of Newfoundland could be a possible reason for this difference.

In phase 1, growth is slow and corresponds to a period of low water temperature (winter and spring). As females begin to migrate towards shallower water, they encounter warmer water (summer and fall). The increased growth rate in phase 2 is therefore most likely related to an increase in water temperature.

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Estimating live standard length of net-caught walleye pollock (*Theragra chalcogramma*) larvae using measurements in addition to standard length*

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Accurate measurement of larval fish lengths from ichthyoplankton samples is critical in the estimation of growth, birthdate and mortality. It is well known that larvae shrink in length 1) when caught in plankton nets (Theilacker, 1980; Hay, 1981), 2) between the time of collection and preservation, and 3) afterwards from the effects of preservation in chemicals or from freezing (Theilacker, 1980; Fowler and Smith, 1983; Yin and Blaxter, 1986). Shrinkage caused by the plankton net (the net can damage a larva's integument; Holliday and Blaxter, 1960) and by delays in preservation can be up to 40% of the initial live length (Hay, 1981). In some cases, measurements of larval body length may be unsuitable for growth estimation (Jennings, 1991). In other cases, algorithms have been developed to estimate live lengths from preserved larvae (Bailey 1982, Hjörleifsson and Klein-MacPhee, 1992; Fey, 1999) and from net-caught and preserved larvae (Theilacker, 1980; Theilacker and Porter, 1995; Fox, 1996). It has also been noted that constant shrinkage-correction factors should be applied cautiously (Pepin et al., 1998).

Shrinkage of the standard length of fish larvae is species-specific (Jennings, 1991; Fey, 1999), size-dependent (Fowler and Smith, 1983; Theilacker and Porter, 1995; Fey, 1999), solution-dependent (Hay, 1982; Tucker and Chester, 1984), and may also depend to some degree on the ambient temperature at the time of preservation (Hay, 1982). Other body measurements may shrink due to effects of collection and preservation. In fact, many corrections for larval shrinkage have been developed with respect to estimating morphometric-dependent condition factors (Theilacker, 1980; McGurk, 1985; Yin and Blaxter, 1986).

Measurement of otoliths has been proposed as a method to correct for shrinkage in the length of fish larvae (Leak, 1986; Radtke, 1989), but this method may not be generally applicable because of systematic variations in the relationship between fish size and otolith size (Neilson and Campana, 1990). In addition, this relationship can be nonlinear and unpredictable. Another approach to obtain shrinkage correction factors is by controlled experiments in which larval shrinkage is recorded from the time of death, or for the period of time larvae are in a plankton net, and again after preservation. The problem with this approach is that during most sampling at sea, the time larvae enter the net to the time of their preservation is unknown and can vary from several minutes to a half hour or more, depending on their depth of capture and the duration of the tow.

In our study, we used measurements of other body parts as ancillary data to estimate live lengths of preserved fish larvae caught at sea. We report on two common preservatives: ethanol and formalin. To avoid the problem of duration of time in the net, shrinkage correction equations were formulated by pooling data of known-length larvae that were treated for varying durations in simulated plankton tows in the laboratory and then preserved. Morphometric measurements were collected on preserved larvae that could be used in equations to obtain more accurate and precise estimates of live length. Rather than use alternative body part measurements as a substitute for length, we hypothesized that other body parts, nonshrinking or otherwise, could be used to correct for shrinkage, in spite of how long larvae had been in nets.

Materials and methods

Rearing protocol

Spawning walleye pollock (*Theragra chalcogramma*) were collected by trawl in the eastern Bering Sea by the NOAA ship *Miller Freeman* during April 1999. Fertilized eggs were transported to the Alaska Fisheries Science Center, Seattle, Washington, where they were incubated at 6°C in the dark in 4-L glass jars filled with 3 L of filtered seawater at a salinity of 33‰. Larvae were reared in 120-L circular, black fiberglass tanks (62 cm diameter, 43 cm deep) filled with 90-L filtered seawater, and maintained at a temperature of 6°C. Two replicate tanks with approximately 1000 eggs each were maintained. A 16:8 hour light:dark cycle was started at hatching and the amount of light at the surface of the water varied from 3.0 to 3.5 µ mol photon/m^2/s. For the first two weeks of feeding, prey consisted of rotifers, *Brachionus plicatilis*, at a concentration of approximately 10/mL, and wild zooplankton (a mixture of *Acartia* sp. copepod nauplii and copepodites, and gastropod and polychaete larvae; at approximately

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1/mL) collected from local lagoons. After two weeks of feeding, *Artemia* sp. brine shrimp (at approximately 10/mL) were added as well. Further details on larval rearing are described in Porter and Theilacker.[1]

Shrinkage experiments

Larval walleye pollock shrinkage was examined for three age groups of larvae (8, 15, and 33 days after hatching) with treatment with preservative only (no net treatment), with 5-minute net treatment and then treatment with preservative, and 15-minute net treatment and then treatment with preservative. The preservatives used were 5% buffered formalin in 33‰ seawater, and 95% ethanol buffered with 0.6 mM sodium carbonate, and 0.6 mM sodium bicarbonate. For each age group, 10 larvae were sampled for each treatment and preservative combination. A pipette was used to place a live larva in seawater on a microscope slide and the larva was quickly recorded on videotape with a Panasonic 5100 digital video camera mounted on a dissecting microscope. Next, the larva was either placed directly into preservative or into the net-treatment apparatus, after which it was preserved. The net-treatment apparatus consisted of a small net submerged in a tank of 6°C seawater. A submersible pump was used to circulate seawater through the net to simulate a plankton tow (Theilacker, 1980). Water flow through the net was adjusted so that a larva would come in contact with the mesh as it might during a tow at sea. One month was chosen as the length of time walleye pollock larvae would remain in preservative before they were recorded on videotape. The length of time for standard length to stabilize in preservative varies depending on the species of fish. For herring (*Clupea harengus*) larvae, standard length was stable after 30 days (Fox, 1996); for red sea bream (*Chrysophrys major*) larvae, most of the shrinkage occurred during the first 2–3 days in presevative (Rosenthal et al., 1978). Optimus version 5.0 image analysis software was used to measure standard length (SL), eye diameter, head length (tip of snout to pectoral fin), and body depth at the anus of both live and preserved larvae.

Otoliths were dissected from the preserved larvae, and maximum diameter of the sagittae were measured at 1000× magnification with an ocular micrometer. Some of the otoliths from larvae preserved in formalin had eroded edges and their diameters could not be measured reliably; therefore, otoliths from formalin-preserved larvae were not used in the shrinkage correction model.

Myomere width measurements taken from formalin preserved larvae were used in the shrinkage correction model. The width of five consecutive myomeres located between the two pigment bands on the tail were measured at 50× with an ocular micrometer and polarized light. For ethanol-preserved larvae, myomere measurements could not be made because the tissue had turned opaque during preservation.

Data analysis

For each preservative, data from all larval ages and shrinkage treatments were pooled and backward stepwise regression (SPSS, Inc., 1998) was used to determine which variables produced the best model for estimating live SL. The data were pooled because the regression is used to estimate live SL of larvae caught at sea and because the amount of time larvae spend in the sampling net is unknown; therefore a general relationship was sought.

For each preservative, the growth rate of walleye pollock larvae between the first (8 days after hatching) and last sampling days (33 days after hatching) was determined by using linear regression. Only the first and last sampling days were used because there is a lag in growth of walleye pollock larvae that occurs for about a week during the transition from endogenous to exogenous feeding (Yamashita and Bailey, 1989). Dunnett's test (Zar, 1996) was used to compare growth rates calculated with live SL (which was considered to be the control) against growth rates calculated from the shrinkage correction models.

Results

Shrinkage with ethanol

Walleye pollock larvae shrank an average of 6% in 95% ethanol when all shrinkage treatments were pooled (Fig. 1A). Larvae used in the shrinkage experiments ranged in live SL from 5.41 to 9.25 mm. The best model to correct for shrinkage contained preserved SL, body depth, and otolith diameter, $r^2 = 0.90$ (Table 1, Fig. 1B). Results indicated that there was borderline multicollinearity in the data (the variance inflation factor for otolith diameter was 4.79), probably due to correlation between preserved SL and otolith diameter. The r^2 with only preserved SL was 0.82. The use of additional variables reduced the mean square error (MSE) by 44% (Table 1).

Residuals from the best model increased as the percentage of shrinkage increased, which suggests that the shrinkage correction model does not work well for larvae that shrink greatly. At largest values of shrinkage, 17% and 19%, the best model underestimated SL by 10%, but at 15% shrinkage the error was 3%.

Shrinkage with formalin

For larvae preserved in 5% formalin, the overall mean shrinkage was 10%. Larvae ranged in live SL from 5.61 to 9.43 mm (Fig. 2A). When compared with the model using only preserved SL (r^2=0.81, MSE=0.112; Table 1), the model containing both preserved SL and body depth at the anus produced the best model for estimating live SL (r^2=0.88, MSE=0.073; Table 1, Fig. 2B).

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[1] Porter, S. M., and G. H. Theilacker. 1996. Larval walleye pollock, *Theragra chalcogramma*, rearing techniques used at the Alaska Fisheries Science Center, Seattle, Washington. AFSC Processed Report 96-06, 26 p. U.S. Dep. Commerce, NOAA, NMFS, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA, 98115.

Table 1

Shrinkage correction equations used to estimate the live standard length (SL) of net-treated and preserved laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae. Best model and model containing only preserved standard length (PSL) are shown. For 95% ethanol, results indicated that there was borderline multicollinearity in the data (see "Results" section). All variables were measured in millimeters. Body depth was measured at the anus. Maximum diameter of the sagitta otolith was used.

95% ethanol (n=91; SL range: 5.41 to 9.25 mm[1])	r^2	MSE
Live SL = 2.18 + 0.464(*PSL*) + 1.71(*body depth*) + 30.53(*otolith diameter*)	0.90	0.073
Live SL = 0.230 + 1.02(*PSL*)	0.82	0.130
5% formalin (n=90; SL range: 5.61 to 9.43[1])		
Live SL = 2.52 + 0.509(*PSL*) + 3.18(*body depth*)	0.88	0.073
Live SL = 1.88 + 0.807(*PSL*)	0.81	0.112

[1] Live standard length of larvae used to formulate regression equations.

Table 2

Growth rates of laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae between the first and last sampling days determined by using live standard length (LSL), and standard length corrected for shrinkage (for shrinkage correction equations see Table 1).

95% ethanol (n=61)	Growth rate (mm/day)	% Error from LSL
Live standard length	0.056	
Standard length corrected with only preserved standard length	0.048	14
Standard length corrected with best model	0.058	4
5% formalin (n=60)		
Live standard length	0.050	
Standard length corrected with only preserved standard length	0.043	14
Standard length corrected with best model	0.045	10

For 5% formalin, residuals for the best shrinkage correction model did not increase as the amount of shrinkage increased; therefore the model worked well for all shrinkage up to the maximum amount of 27%.

Growth rates

Growth rates were calculated with live SL, corrected SL from the preserved SL only models, and corrected SL from the multivariate models. For each preservative there was no significant difference between the growth rate for live SL and growth rates calculated with the models (Dunnett's test, $P>0.05$). Although the growth rates were not significantly different, growth rates calculated from the multivariate models were more similar to growth rates calculated from live SL (Table 2); therefore additional morphometric variables improve growth rate estimates over using preserved SL alone.

Discussion

Fish larvae shrink considerably when they are caught in nets and preserved. Shrinkage during collection is most likely caused by the plankton net, which can damage the larva's integument (Holliday and Blaxter, 1960). Shrinkage also varies between preservatives because of the differing ionic strengths of these solutions (Parker, 1963; Hay, 1982; Tucker and Chester, 1984). We found that shrinkage of walleye pollock larvae was greater in 5% formalin than in 95% ethanol—a finding similar to results from other species (Bailey, 1982; Fey, 1999). Often only preserved SL has been used to correct for shrinkage in length (Theilacker and Porter, 1995; Fox, 1996; Kristoffersen and Gro Vea Salvanes, 1998), although other measurements, such as otolith size, have been suggested for use to correct for larval fish shrinkage (Leak, 1986; Radtke, 1989,1990). By using measurements that are easily made and readily adapted to

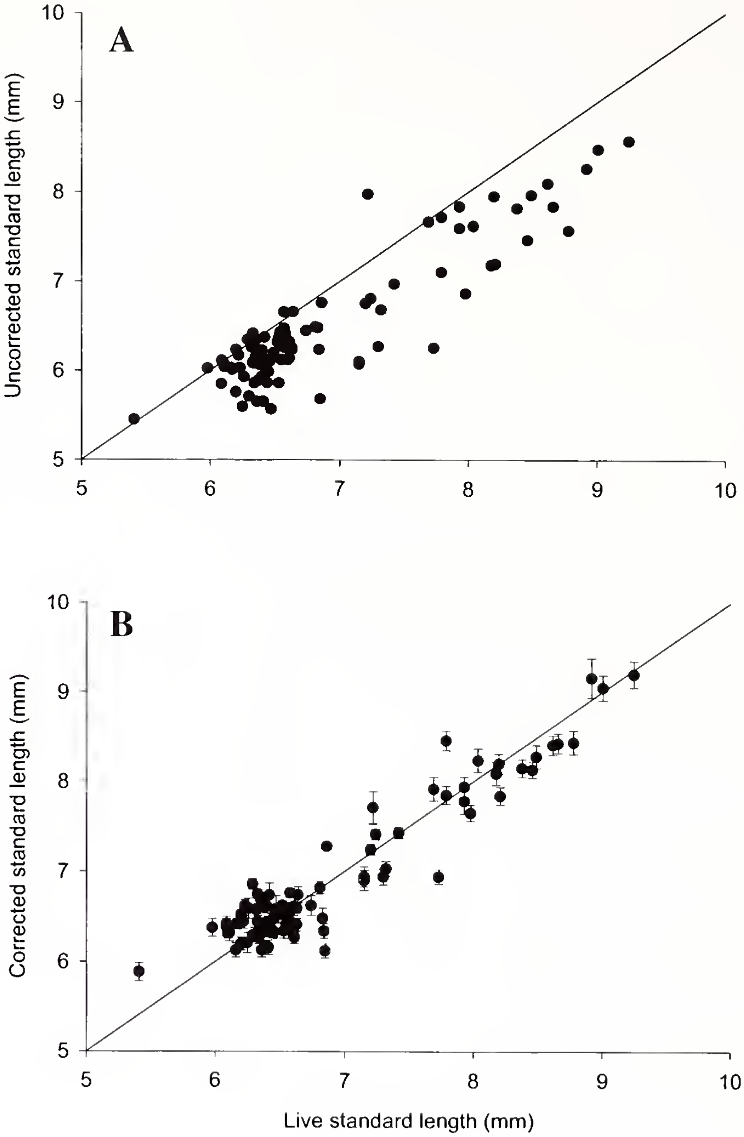

Figure 1

Uncorrected (**A**) and corrected (**B**) standard length determined by using best shrinkage correction model, see Table 1) for laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae subjected to various shrinkage treatments and preserved in 95% ethanol (n=91). The 95% confidence limits are shown for each predicted value, and the diagonal line shows a 1:1 ratio.

routine data collection, we have shown improved accuracy of shrinkage correction models by use of measurements in addition to preserved SL: for 5% formalin, 7% more of the variance was explained by adding body depth; and for 95% ethanol, 8% more of the variance was explained by including body depth and otolith diameter. For 95% ethanol, the shrinkage correction model formulated in our study may significantly underestimate SL when shrinkage is high, but because there were only two larvae in this category, results were inconclusive.

Shrinkage correction models are usually formulated by using fed, laboratory-reared larvae (Jennings, 1991; Theilacker and Porter, 1995; Fox, 1996), but individual larvae response to handling and preservation can vary significantly (Pepin et al., 1998). These correction models were developed to allow more accurate calculation of the growth rate of fish larvae in the sea and to apply laboratory-derived indices to the field. We have shown factors in addition to preserved SL that may improve the accuracy of live-length estimates for walleye pollock.

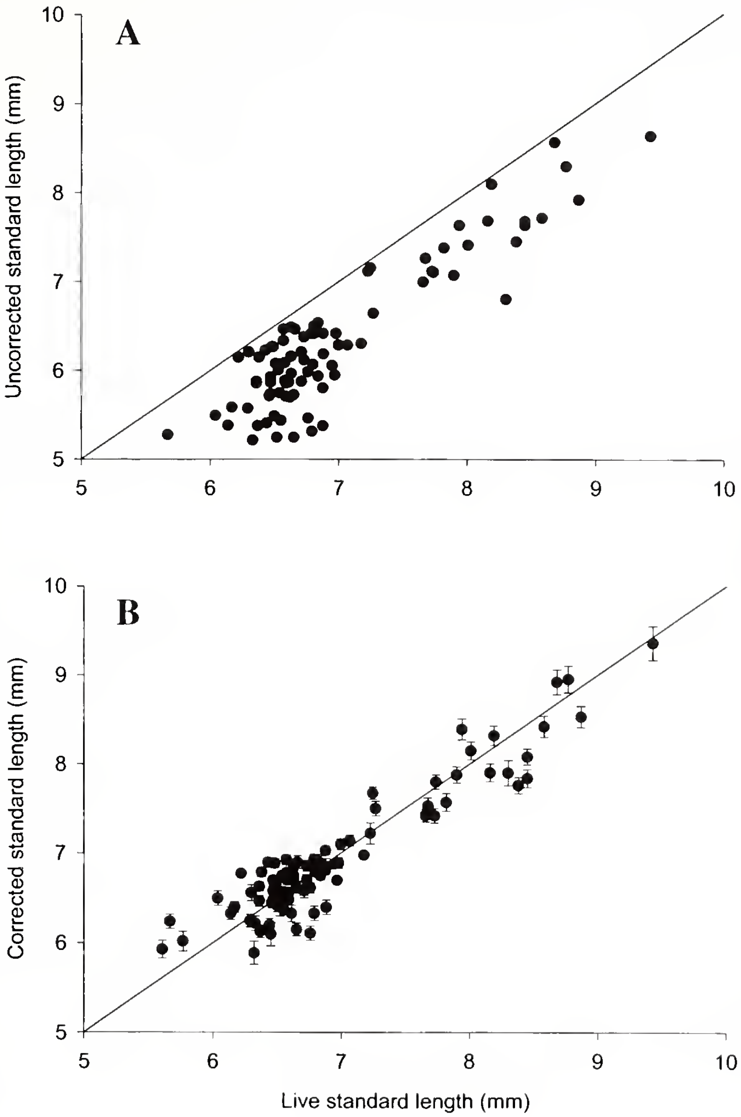

Figure 2

Uncorrected (**A**) and corrected (**B**) standard length determined by using best shrinkage correction model, see Table 1) for laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae subjected to various shrinkage treatments and preserved in 5% formalin (n=90). The 95% confidence limits are shown for each predicted value, and the diagonal line shows a 1:1 ratio.

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Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis

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The albacore (*Thunnus alalunga*) is a highly migratory large pelagic tuna, common from tropical to temperate areas of all oceans, including the Mediterranean Sea. Although albacore populations of each ocean or each hemisphere have been managed separately, relationships between albacore populations of northern and southern hemispheres within an ocean are controversial. Differences in morphometry, movement, and catch statistics of albacore between northern and southern hemispheres within Atlantic and Pacific Oceans have been reported (Kurogane and Hiyama, 1958; Ishii, 1965; Nakamura, 1969). Examining mtDNA variation, Chow and Ushiama (1995) detected very little genetic difference between samples from northern and southern hemispheres. They proposed that minor gene flow may have occurred between albacore populations of northern and southern hemispheres—enough to prevent genetic differentiation. However, it is also possible that insufficient time has elapsed since population subdivision for mtDNA genotypic rearrangement. Because all tuna species of the genus *Thunnus* are thought to be phylogenetically new (Chow and Kishino, 1995), use of highly variable genetic markers may be necessary to investigate genetic differentiation between stocks.

Recently, Takagi et al. (1999) isolated four microsatellite loci from Pacific northern bluefin tuna (*Thunnus thynnus orientalis*) and demonstrated successful cross-species amplification of homologous microsatellites in other tuna species. In our study, we used these microsatellite primers to evaluate genetic variation within and between albacore samples from the Atlantic and the Pacific Oceans and present genetic evidence of population structuring between and within ocean samples of albacore.

Materials and methods

Five albacore samples were drawn from archival stock materials in the National Research Institute of Far Seas Fisheries laboratory. One sample each came from the Northwest Pacific (NW Pacific; Japan), Southwest Pacific (SW Pacific; Australia), Southeast Pacific (SE Pacific; Chile and Peru), and two came from the Northeast Atlantic (NE Atlantic; Biscay Bay) and the Southwest Atlantic (SW Atlantic; Brazil). These samples were derived from the same sample lots used by Chow and Ushiama (1995). Nucleotide sequences of the four primer sets, PCR amplification conditions needed to amplify the four microsatellite loci (_Ttho-1*_, _-4*_, _-6*_ and _-7*_) developed for Pacific northern bluefin tuna (*T. thynnus orientalis*), and electrophoresis procedures can be found in Takagi et al. (1999). Differentiation of allele frequencies between and among samples was estimated by a fixation index (F_{ST}) with Arlequin version 1.1 (Schneider et al., 1997).

Results

Alleles observed in each locus were as follows: 9 in _Ttho-1*_, 29 in _Ttho-4*_, 31 in _Ttho-6*_, and 18 in _Ttho-7*_ (Table 1). All four sets of PCR primers successfully amplified scorable microsatellite loci for all samples. Observed heterozygosity (H_0) ranged from 0.391 to 0.914 at _Ttho-1*_, from 0.688 to 0.886 at _Ttho-4*_, from 0.548 to 0.884 at _Ttho-6*_, and from 0.857 to 1 at _Ttho-7*_. We found no substantial discrepancy between observed and expected number of genotypes for any locus.

All samples except NE Atlantic shared the most common alleles for all loci, whereas the NE Atlantic sample shared the most common alleles only within _Ttho-1*_. The NE Atlantic sample also did not share the second most common allele with the other samples for all loci. F_{ST} among all five

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Table 1

Allele frequency and genetic variability for four microsatelite loci surveyed for albacore used in this study.

Locus	Allele (base pairs)	NW Pacific	SW Pacific	SE Pacific	SW Atlantic	NE Atlantic
Ttho-1*	172	0.000	0.000	0.021	0.000	0.000
	174	0.000	0.016	0.000	0.000	0.000
	176	0.075	0.065	0.043	0.054	0.065
	178	0.000	0.097	0.096	0.000	0.032
	180	0.234	0.177	0.202	0.141	0.145
	182	0.521	0.452	0.436	0.685	0.516
	184	0.149	0.161	0.160	0.098	0.210
	186	0.000	0.032	0.043	0.022	0.032
	188	0.021	0.000	0.000	0.000	0.000
No. of samples		47	31	47	46	31
No. of allele		5	7	7	5	6
Effective no. of alleles[1]		2.82	3.62	3.70	1.99	2.96
Observed heterozygosity (H_o)		0.659	0.871	0.914	0.391	0.645
Expected heterozygosity (H_e)		0.645	0.724	0.730	0.498	0.662
Ttho-4*	134	0.000	0.000	0.000	0.000	0.032
	136	0.000	0.016	0.011	0.000	0.096
	138	0.011	0.016	0.033	0.011	0.065
	140	0.043	0.031	0.067	0.011	0.452
	142	0.032	0.031	0.167	0.000	0.032
	144	0.085	0.094	0.100	0.102	0.065
	146	0.383	0.313	0.178	0.261	0.048
	148	0.032	0.078	0.089	0.080	0.097
	150	0.011	0.094	0.056	0.023	0.032
	152	0.053	0.047	0.067	0.102	0.048
	154	0.096	0.031	0.044	0.046	0.032
	156	0.011	0.000	0.011	0.068	0.000
	158	0.032	0.047	0.000	0.057	0.000
	160	0.043	0.047	0.044	0.046	0.000
	162	0.021	0.031	0.033	0.034	0.000
	164	0.011	0.078	0.044	0.023	0.000
	166	0.053	0.000	0.000	0.023	0.000
	168	0.021	0.031	0.011	0.011	0.000
	170	0.000	0.000	0.022	0.011	0.000
	174	0.000	0.000	0.011	0.023	0.000
	176	0.000	0.000	0.000	0.023	0.000
	178	0.000	0.000	0.011	0.023	0.000
	180	0.032	0.000	0.000	0.000	0.000
	182	0.011	0.000	0.000	0.000	0.000
	184	0.011	0.000	0.000	0.000	0.000
	186	0.000	0.016	0.000	0.000	0.000
	190	0.011	0.000	0.000	0.000	0.000
	202	0.000	0.000	0.000	0.011	0.000
	232	0.000	0.000	0.000	0.011	0.000
No. of samples		47	32	45	44	31
No. of allele		20	16	18	21	11
Effective no. of alleles[1]		5.62	7.14	10.20	8.85	4.17
Observed heterozygosity (H_o)		0.851	0.688	0.844	0.886	0.710
Expected heterozygosity (H_e)		0.822	0.860	0.902	0.887	0.760
Ttho-6*	127	0.000	0.000	0.000	0.012	0.000
	129	0.000	0.000	0.000	0.012	0.000
	131	0.011	0.032	0.000	0.000	0.000
	133	0.011	0.000	0.000	0.000	0.000
	135	0.033	0.113	0.047	0.093	0.000

continued

Table 1 (continued)

Locus	Allele (base pairs)	NW Pacific	SW Pacific	SE Pacific	SW Atlantic	NE Atlantic
Ttho-6* (continued)	137	0.100	0.000	0.023	0.105	0.000
	139	0.056	0.016	0.093	0.047	0.000
	141	0.478	0.629	0.233	0.349	0.083
	143	0.067	0.048	0.081	0.105	0.017
	145	0.167	0.113	0.163	0.128	0.050
	147	0.067	0.048	0.093	0.058	0.300
	149	0.000	0.000	0.081	0.047	0.083
	151	0.011	0.000	0.023	0.012	0.017
	153	0.000	0.000	0.012	0.000	0.033
	155	0.000	0.000	0.035	0.000	0.050
	157	0.000	0.000	0.000	0.012	0.033
	159	0.000	0.000	0.000	0.023	0.050
	161	0.000	0.000	0.012	0.000	0.083
	163	0.000	0.000	0.023	0.000	0.000
	165	0.000	0.000	0.000	0.000	0.033
	167	0.000	0.000	0.023	0.000	0.050
	169	0.000	0.000	0.000	0.000	0.033
	171	0.000	0.000	0.012	0.000	0.017
	177	0.000	0.000	0.012	0.000	0.000
	183	0.000	0.000	0.000	0.000	0.017
	185	0.000	0.000	0.000	0.000	0.017
	187	0.000	0.000	0.012	0.000	0.000
	189	0.000	0.000	0.012	0.000	0.000
	191	0.000	0.000	0.012	0.000	0.000
	199	0.000	0.000	0.000	0.000	0.017
	201	0.000	0.000	0.000	0.000	0.017
No. of samples		45	31	43	43	30
No. of allele		10	7	19	13	19
Effective no. of alleles[1]		3.58	2.34	8.47	5.65	7.87
Observed heterozygosity (H_o)		0.689	0.548	0.884	0.884	0.867
Expected heterozygosity (H_e)		0.721	0.573	0.882	0.823	0.873
Ttho-7*	182	0.000	0.031	0.000	0.000	0.000
	188	0.083	0.109	0.032	0.000	0.017
	190	0.024	0.000	0.021	0.010	0.033
	192	0.024	0.063	0.075	0.052	0.000
	194	0.321	0.203	0.287	0.250	0.217
	196	0.012	0.063	0.096	0.146	0.117
	198	0.024	0.078	0.053	0.125	0.317
	200	0.083	0.172	0.074	0.135	0.050
	202	0.012	0.031	0.053	0.042	0.083
	204	0.024	0.000	0.011	0.031	0.017
	206	0.012	0.016	0.043	0.000	0.017
	208	0.060	0.078	0.032	0.031	0.050
	210	0.131	0.063	0.075	0.042	0.017
	212	0.107	0.000	0.021	0.063	0.067
	214	0.024	0.031	0.053	0.031	0.000
	216	0.024	0.031	0.043	0.010	0.000
	218	0.024	0.031	0.032	0.010	0.000
	224	0.000	0.000	0.000	0.010	0.000
No. of samples		42	32	47	48	30
No. of allele		16	14	16	15	12
Effective no. of allele		6.49	8.93	8.06	7.63	5.59
Observed heterozygosity (H_o)		0.857	0.906	0.872	0.896	1.000
Expected heterozygosity (H_e)		0.846	0.888	0.876	0.869	0.821

[1] Effective number of alleles was calculated with the equation $1/(1-H_e)$.

samples deviated considerably from zero (F_{ST}=0.018 to 0.070, $P<0.001$) for all loci. Significant departures of F_{ST} from zero were observed for three loci (*Ttho-1**, *Ttho-4**, and *Ttho-6**) among all samples except the NE Atlantic sample (F_{ST} =0.013 to 0.085, $P<0.005$) and among the three Pacific samples (F_{ST}=0.018 to 0.074, $P<0.001$). There were also significant differences in allele frequencies between some pairwise comparisons (Table 2). At *Ttho-1**, the SW Pacific sample showed significant difference from all other samples (all $P<0.001$), and there was also a significant difference between the SE Pacific and SW Atlantic samples (P=0.003). The NE Atlantic sample was significantly heterogeneous in comparison with all other samples in *Ttho-4** (all $P<0.001$). Difference between the NW and SE Pacific samples was also significant in this locus (P=0.001). In *Ttho-6** the NE Atlantic sample was again significantly heterogeneous in comparison with all other samples (all $P<0.001$). Furthermore, there were also significant differences between the NW and SE Pacific, between the SW and SE Pacific, and between the SW Pacific and SW Atlantic samples in this locus (all $P<0.001$). In *Ttho-7** the NE Atlantic sample showed significant differences from all three Pacific samples ($P<0.001$ for the NW and SW Pacific, P=0.003 for the SE Pacific) but not from the SW Atlantic sample (P=0.017).

Table 2

Pairwise comparison of F_{ST} between five albacore samples.

	NW Pacific	SW Pacific	SE Pacific	SW Atlantic
Ttho-1*				
SW Pacific	0.1401			
SE Pacific	0.005	0.099[1]		
SW Atlantic	0.018	0.249[1]	0.0461	
NE Atlantic	–0.004	0.154[1]	–0.003	0.023
Ttho-4*				
SW Pacific	0.003			
SE Pacific	0.032[1]	0.012		
SW Atlantic	0.006	–0.001	0.017	
NE Atlantic	0.151[1]	0.131[1]	0.094[1]	0.131[1]
Ttho-6*				
SW Pacific	0.020			
SE Pacific	0.038[1]	0.100[1]		
SW Atlantic	0.006	0.052[1]	0.010	
NE Atlantic	0.135[1]	0.208[1]	0.0431	0.086[1]
Ttho-7*				
SW Pacific	0.012			
SE Pacific	0.006	0.003		
SW Atlantic	0.020	0.006	0.001	
NE Atlantic	0.592[1]	0.042[1]	0.040[1]	0.017

[1] Significant difference ($P<0.005$).

Discussion

Because the number of alleles observed in microsatellite loci is usually large and the frequency of each allele may be low, a large sample size is necessary for satisfying subsequent statistic analyses. Ruzzante (1997) showed that a sample size of $50 < n < 100$ individuals is generally satisfactory, although this size depends on allele number and frequency. Size of our samples ranged from 32 to 48, close to the lower margin of this threshold. Nevertheless, the distinct status of the Northeast Atlantic albacore sample from others is obvious. Although our study shared the same sample lots with Chow and Ushiama (1995), their mtDNA analysis revealed much less genetic differentiation between samples from the North and South Atlantic. MtDNA analysis is thought to be a more effective indicator for population subdivision than nuclear DNA (Nei and Li, 1979). But for albacore in the Atlantic, this is obviously not the case. No selection toward microsatellite alleles is obvious; therefore, differences in evolutionary rate between mtDNA and nDNA may explain differences in allele frequency distribution in the Atlantic albacore. Because mtDNA variation observed by Chow and Ushiama (1995) was much lower than the microsatellite DNA variation observed in our present study, there might have been insufficient elapsed time for unique mitochondrial genotypes to have arisen within the existing stocks. Thus, the rapidly evolving microsatellites appear to reflect albacore population subdivision.

MtDNA analyses have also failed to detect genetic difference between samples from northern and southern hemispheres within the Pacific (Chow and Ushiama, 1995). In contrast, present microsatellite analysis detected significant differences among three Pacific samples, clearly indicating that microsatellites appear to be more sensitive and powerful in detecting more subtle signals of genetic differentiation in albacore samples than mtDNA analysis. These results support several ecological and morphometric studies (Nakamura, 1969; Lewis, 1990) which assumed negligible migration of albacore across the equator in the Pacific. Separate North and South Pacific albacore stocks is a reasonable assumption because two major spawning grounds confined in the western to mid tropical Pacific are spatiotemporarily separated (Nishikawa et al., 1985). However, it is difficult to explain genetic differentiation between southwest and southeast Pacific albacore samples because no major spawning ground of albacore has been determined in the southeast Pacific. Microsatellite analysis of a sample from a different year class and a larger sample size is necessary to better define the observed genetic differences among the Pacific samples and the South Atlantic sample.

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Erratum

Fishery Bulletin **99(3):399–409**

Colura, Robert L., and Britt W. Bumguardner

Effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch

Erratum. Page 400, left column, last four lines.

The sentence "For each species, probit analysis was conducted on untransformed data to determine the time at which 50% of the test animals exposed to the test salinity died (LE_{50}) (Sokal and Rohlf, 1981)" should read

"For each species (48 hour), probit analysis was conducted on untransformed data to determine the time at which 50% of the test animals exposed to the test salinity died (LE_{50}) (Sokal and Rohlf, 1981)."

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